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RESEARCH ARTICLE

Categorization of Diverse and Stable Extant Cultivars of Brinjal by using Pheno-morphometric DUS Characters

B. Singh*, T. Chaubey, S. Pandey, RK Singh, DK Upadhyay, A Jha and SD Pandey

Abstract

A total of 81 extant cultivars of Brinjal (*Solanum melongena* L.) were evaluated for 47 botany based morphometric descriptors from various parts of a plant viz., seedling, stem, leaves, flowers and fruits, etc., as well as characterized on the basis of distinctiveness, uniformity and stability (DUS) test guidelines. All the cultivars were analyzed for diversity and stability. In the present study, all the cultivars exhibited distinct and uniform characters. The economic importance of all the botanical traits were also discussed during the study. The cluster analysis grouped the varieties in four major and 10 sub-clusters, indicating a high level of genetic diversity within 81 extant cultivars. The stability analysis indicate a significant interaction between the cultivars and environment for yield contributing characters. These yield related traits showed stability for most cultivars in different environments. The characterized morphometric characters in this study would be helpful for creating a database for brinjal while the genetically stable cultivars can be cultivated in any climatic region and be utilized for the improvement programme of brinjal.

Keywords: Brinjal, Morphological yield, Distinctiveness, Genetic diversity, Stability.

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Introduction

Brinjal (*Solanum melongena* L.) is an old vegetable of India but it was domesticated from Indo-Burma region to Northern Thailand, Laos, Vietnam and Southwest China (Vavilov, 1928; Daunay and Janick, 2007). A large number of advanced genetic resources of brinjal have been cultivated in India. Brinjal is famous for its different and diverse vegetative, floral and fruit characters like shape, size, and color and its various use as fresh, dried or pickled (Kumar *et al.*, 2008; Oladosu *et al.*, 2021). The color of leaves, flowers and fruits in a crop varies depending on the availability of different color pigmentations. The green color of the leaves and fruits of brinjal is due to the availability of chlorophyll color pigments while the purple color in seedling, stems, leaves, flowers and fruits of brinjal has been indicated to the availability of anthocyanin color pigments (Polignano *et al.*, 2010; Chaudhary and Mukhopadhyay, 2012; Younas *et al.*, 2022). It has been cited that the presence of anthocyanins in vegetable will be beneficial for human and animal health due to presence of antioxidant ability (Vyas *et al.*, 2009; Chaudhary and Mukhopadhyay, 2012; Gurbuz *et al.*, 2018). Brinjal leaves are frequently used in southern countries for soup and other delicious menu like brinjal fruits (Kouassi *et al.*, 2014). Fruit size, shape, color, glossiness and earliness of brinjal are most important characteristics because they are directly related to demands for commercialization at different locations and interest of people (Adeniji *et al.*, 2013). The fruiting pattern depends on the number of flowers and plant growth habit (Kouassi *et al.*, 2014).

The evaluation of genetic diversity and relationships of the cultivated species of vegetable crop facilitates the establishment of conservation and utilization in breeding programmes (Ali *et al.*, 2011). Earlier, it has been analyzed that the genetic diversity within a large collection of germplasm may be helpful to make a strategy for a current and future breeding program (Caguiat and Hautea, 2014). However, the study of the description and classification of morphological characteristics is the first step of maintenance breeding which is essential to describe the distinctiveness and stability in the cultivars (PPV&FRA, 2009). Stable genotype is very much important and desirable to identify the suitable genotype for cultivation in different environments (Eberhart and Russell, 1966). The higher magnitude of the genotype and environmental interaction indicates the stability in morphological and yield related traits against different environments (Mehta *et al.*, 2011; Sivakumar *et al.*, 2015; Bhushan and Samnotra, 2017). It can't be ignored that morphological data may be dubious for taxonomic reliability because of environmental interference (Oladosu *et al.*, 2021; Younas *et al.*, 2022). Previously, limited information has been published regarding the characterization of huge morphological traits of brinjal along with their economic importance on a single platform. This study may be more useful for students, researchers and industrialists to improve their knowledge about brinjal. Therefore, this study aimed to categorize the extant cultivars of brinjal on the basis of distinct pheno-morphometric traits for analysing the distinctness, uniformity and stability test and to identify the divers and stable cultivars for developing new varieties of brinjal.

Materials And Methods

Plant Materials and Transplanting

A total of 81 extant cultivars of brinjal developed and received from ICAR Institutes, CAUs (Central Agricultural Universities) and SAUs (State Agricultural Universities) of India, while, two cultivars 'Uttara' and 'Nurki' were developed in Bangladesh and Nepal, respectively (Table S1). All the extant cultivars of brinjal were evaluated in the experimental farm of ICAR-Indian Institute of Vegetable Research, Varanasi for analysing the distinctiveness, uniformity and stability. Total 150 brinjal plants were transplanted in a randomized block design in three replications (50 plants in each replication) at a spacing of 60 cm (line to line) and 45 cm (plant to plant) during the *khari*f season of years 2013, 2014, 2015 and 2016. All the agronomical and cultural practices were followed for maintaining healthy crops.

Data Observation

Observation on 47 botany based morphometric characters were recorded as DUS guidelines of brinjal were recorded (PPV&FRA 2009). Data were recorded from each replication

(avoiding the border rows) at specified stages of the crop growth period when the characters had their full expression. For the assessment of color characteristics, the Royal Horticultural Society (RHS, 2001) color chart was used. All observations on the stem, leaves, flowers were recorded from the first inflorescence to first harvesting whereas, observations on fruits were recorded on the commercial and physiological maturity stages. The data was observed for all the 47 morphometric traits from the following plant parts e.g., seedling, stem, leaf, flower and fruits within four assessing groups *viz.*, MG (measurement by a single observation on a group of plants or parts of plants), MS (measurement on a number of individual plant or parts of plants), VG (Visual assessment by a single observation on a group of plants or parts of plants), VS (Visual assessment by observations on individual plant or parts of plants) as discussed in DUS guideline of brinjal (Table S2-S4).

Leaf size area index: It was calculated by dividing leaf length from leaf width. Leaf size was measured by scale for <1 cm= small, 1–2 cm= medium and >2 cm= large, these scales indicated that small=<10 cm, medium=10–20 cm and large=>20 cm of leaf length and width (Snelgar and Martin, 1997; Singh *et al.*, 2015).

Fruit size index: It was calculated by dividing fruit length from fruit diameter. Fruit size was measured by scale for <1 cm= short and small, 1–2 cm= medium and >2 cm= long and large, these scales indicated that short=<10 cm, medium=10–20 cm and long=>20 cm for fruit length and small=<5 cm, medium=5–10 cm and large=>10 cm for fruit diameter (Hjeltnes, 1994; Oladosu *et al.*, 2021).

Diversity Analysis

For genetic diversity a dendrogram (cluster) constructed by using standardized morphometric characters of all 81 extant cultivars of brinjal. The dendrogram was constructed based on the euclidean distance coefficient and unweighted pair-group method of arithmetic means (UPGMA) using the SAHN program in numerical taxonomy and multivariate analysis system (NTSYS-PC software) version 2.11s (Rohlf, 2005).

Stability Analysis

Among the 81 extant cultivars of brinjal 48 cultivars were selected on the basis of high growth, better yield and earliness for the stability test analysis over four environments according to the stability model of Eberhart and Russell (1966). Six characters *viz.*, fruit length (cm), fruit diameter (cm), number of fruit/plant, plant height (cm), time of physiological ripeness (days) and fruit yield/plant (kg) were used for identifying stable genotypes from 48 extant cultivars. The data was analysed for variance to test the significance of genotypes $\times$ environmental interaction. The model involved the estimation of mean (m), regression coefficient (b) and deviation from regression (s^2di).

Results

Characterization of Pheno-Morphometric Traits

Seedling, Stem and Leaves

At the seedling stage the anthocyanin coloration of hypocotyls were absent in 23 extant cultivars (29.63%), while 58 extant cultivars showed presence of anthocyanin coloration with different color intensity like weak (18), medium (20), strong (17) and very strong (3) in the proportionate ratio of 20.99, 24.69, 20.99 and 3.70%, respectively (Table S2). However, while observing the active vegetative phase of plants, the anthocyanin coloration of stem (Figure 1) was absent in 35 extant cultivars and present in 46 extant cultivars, which were categorized in order of weak (25.93%), medium (20.99%), strong (2.47%) and very strong (7.41%) for 22, 17, 2 and 5 extant cultivars (Table S2). The considerable variation was observed in stem pubescence (Figure 1) or hairs which were expressed as weak, medium and strong on 30 (37.04%), 38 (46.91%) and 13 (16.05%) extant cultivars, respectively (Table S2).

Leaf size area index was calculated by dividing the leaf blade length by leaf blade width. No extant cultivars were found to be having small leaves (<1.0 cm = <10.00 cm), while, five cultivars (6.17%) were having large leaves (>2.0 cm ≤ 20.00 cm). However, 76 (93.83%) extant cultivars expressed to be having medium leaf size in the range of 1 to 2 cm (10–20 cm) of leaf length and width (Table S2; Figure 2). In case of leaf margin, the extant cultivars were categorized into three groups comprising 30 extant cultivars as entire (37.04%), 37 as dentate (45.68%) and 14 as sinuate (17.28%). Among the cultivars, 46 (56.79%) exhibited leaf blistering character. In comparison, in 35 (43.21%) cultivars had absent blistering whereas, the intensity of spininess (Figure 2) on leaves were absent in 75 (92.59%) extant cultivars. In contrast, the leaf spiny was weak (<5) in JBGR-1 and JBL-03-03, medium (5–10) in Swarna Abhilamb and strong (>10) in 3 cultivars namely Swarna Ajay, Swarna Shobha and Swarna Shyamali, respectively. In the present study, it was also observed that the leaf blade color and leaf vein color of extant cultivars expressed themselves as green (60 and



Figure 1: Anthocyanin coloration and presence of pubescence on the stem of brinjal.

10 cultivars, respectively) and purple (21 and 71 cultivars, respectively) color in the intensity of light, medium and dark (Table S2; Figure 2).

Flowers and Fruits

Among the 81 extant cultivars, 51 (62.96%) cultivars expressed 1 to 3 number of flowers and the remaining 30 (37.04%) cultivars had more than three (>3) flowers in each inflorescence (Table S3). In case of flower size, 27.16 (22 cultivars), 44.44% (36 cultivars), and 28.40% (23 cultivars) showed small, medium and large flower size, respectively (Figure 3). In the present study, the flower color of each cultivar indicated high variability which was divided into four different colors i.e. greenish white, light purple, purple and dark purple (Figure 3). Four (4.94%) cultivars like, 'Arka Shirish', 'Arka Shree', 'green long cluster' and 'Swarna Prabha' exhibited greenish white flower color, while 19 (23.46%) cultivars showed light purple, 39 (48.15%) cultivars had purple and the rest 19 (23.46%) cultivars expressed as dark purple flower color. Another finding includes the flowering times in cultivars which were classified into three categories early (<60 days), medium (60–80 days) and late (>80 days) from days after seed sowing. Among the cultivars, 63 (77.78%) were recorded for medium flowering time while the rest 18 cultivars (22.22%) were expressed as early flowering time. None of the cultivar recorded for late (>80 days) flowering (Table S3).

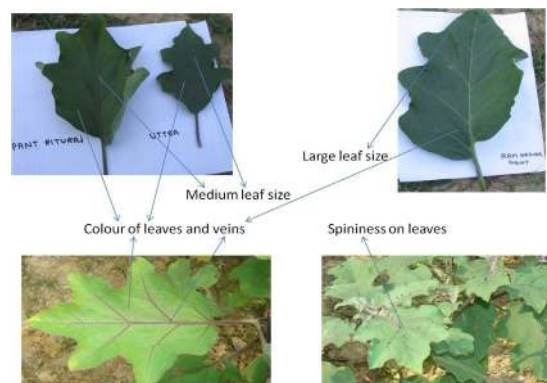


Figure 2: Color of leaf blade and vein, margin shape, leaf size and spininess nature of the leaves of brinjal.

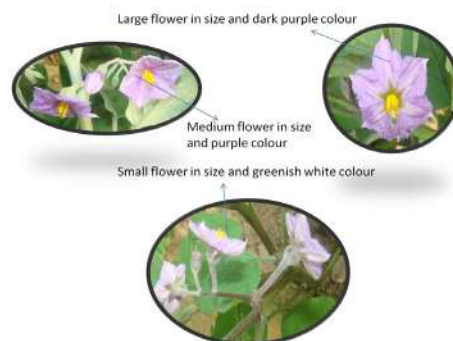


Figure 3: Size, color and nature of the flowers in brinjal.

Table 1: Analysis of variance for stability analysis in 48 extant cultivars of brinjal under different environments for 6 yield related traits

Source of variation	DF	FL	FD	NFPP	PH	TPR	FYPP
Variety	47	71.15**	26.58**	104.70**	1,193.22**	92.99**	0.75**
Environment	3	85.53**	1.98**	415.78**	111.34**	74.26**	0.67**
Var. × Environ.	141	2.02**	0.13*	4.51**	8.43**	9.33*	0.57**
Env+Var × Env	144	1.80**	0.05**	13.08**	2.35**	1.87**	0.02**
Env (Linear)	1	256.59**	5.95**	1,247.34**	334.01**	222.77**	2.02**
Env × Var(Lin)	47	1.02**	0.10*	3.69**	5.06**	5.74**	0.54**
Pooled Deviation	96	0.11	0.26	6.29	0.21	0.12	0.15
Pooled Error	376	0.15	0.14	3.74	15.44	11.30	0.21

*,**Significance Levels at $P < 0.05$, $P < 0.01$; DF= Degree of freedom; FL=Fruit length (cm); FD=Fruit diameter; NFPP=Number of fruit/plant; PH=Plant height; TPR=Time of physiological ripeness (days after fruit set); FYPP=Fruit yield/plant.

Table 2: Stability analysis in 48 extant cultivars of brinjal under different environments for 6 yield related traits

Genotypes	FL			FD			NFPP			PH			TPR			FYPP		
	m	b	S ² di	m	b	S ² di	m	b	S ² di	m	B	S ² di	m	b	S ² di	m	b	S ² di
Annamali	11.14	1.00	-0.05	4.13	1.06	-0.01	19.83	0.67	25.45	94.33	1.03	-5.15	71.33	1.25	-3.74	2.61	1.19	-0.003
Arka Keshav	15.44	1.00	-0.05	5.60	1.06	-0.01	31.25	0.96	-0.90	101.91	0.94	-5.14	69.25	1.37	-3.73	2.08	1.05	-0.003
Arka Neelkanth	11.94	1.00	-0.05	5.60	1.06	-0.01	31.42	1.09	0.70	96.93	0.74	-5.11	73.00	1.25	-3.74	2.55	1.25	-0.003
Arka Nidhi	9.18	1.00	-0.05	10.36	0.18	-0.01	26.50	1.23	11.60	78.13	1.03	-5.15	70.66	1.04	-3.77	2.13	0.93	-0.003
Arka Sheel	13.68	1.00	-0.05	4.50	1.06	-0.01	20.83	0.87	2.69	101.78	0.59	-4.82	71.00	1.25	-3.74	1.88	0.88	-0.004
Arka Shirish	14.54	1.00	-0.05	6.50	1.06	-0.01	23.00	1.15	4.47	82.63	1.03	-5.15	70.67	0.79	-3.56	1.95	0.82	-0.003
Arka Shree	10.08	1.00	-0.05	7.97	1.06	-0.01	18.83	0.87	2.69	103.33	0.64	-5.14	58.58	1.37	-3.73	2.13	0.99	-0.003
Aruna	5.81	1.00	-0.05	4.97	1.06	-0.01	21.25	1.22	10.21	55.36	1.03	-5.15	61.75	0.68	-3.46	1.79	0.90	-0.003
Azad Brinjal-1	9.11	1.00	-0.05	8.53	1.06	-0.01	16.83	0.72	17.86	92.83	1.03	-5.15	70.67	0.44	-3.66	2.63	1.15	-0.004
Azad Brinjal-2	22.11	1.00	-0.05	3.77	1.06	-0.01	25.17	1.13	2.72	128.63	1.03	-5.15	59.92	1.37	-3.73	2.00	0.97	-0.004
Azad Brinjal-3	16.94	1.00	-0.05	4.60	1.06	-0.01	19.33	1.31	21.57	109.73	1.03	-5.15	69.67	1.10	-3.58	2.32	1.09	-0.004
Azad Brinjal-4	10.21	1.00	-0.05	10.50	1.06	-0.01	10.08	0.78	10.15	128.56	1.03	-5.15	69.58	1.37	-3.73	2.09	0.95	-0.003
Bhagyamati	9.94	1.00	-0.05	4.93	1.06	-0.01	16.33	1.15	4.47	81.19	1.03	-5.15	70.83	0.41	-3.66	2.12	0.82	-0.003
BR-14	9.28	1.00	-0.05	8.73	1.06	-0.01	11.83	0.98	-1.09	87.09	1.03	-5.15	71.33	1.25	-3.74	2.88	1.40	-0.003
CH-1045	9.81	1.00	-0.05	7.23	1.06	-0.01	13.17	0.92	0.17	89.56	1.03	-5.15	60.25	1.37	-3.73	2.61	1.05	-0.003
CHBR-2	5.98	1.00	-0.05	7.77	1.06	-0.01	16.33	0.85	4.43	86.39	1.03	-5.15	68.85	0.81	-3.36	2.57	1.15	-0.004
DBR-3	6.91	1.00	-0.05	5.60	1.06	-0.01	20.67	1.10	1.30	88.53	1.03	-5.15	72.58	0.91	-3.64	1.75	0.88	-0.004
DBR-8	18.48	1.00	-0.05	8.70	1.06	-0.01	16.67	1.15	4.47	92.23	1.03	-5.15	71.08	0.53	-3.70	1.99	0.90	-0.003
DRNKV-2-29	13.68	1.00	-0.05	9.67	1.06	-0.01	12.33	0.90	1.27	81.23	1.03	-5.15	72.58	1.37	-3.73	1.93	0.90	-0.003
GBL-1	17.84	1.00	-0.05	4.83	1.06	-0.01	16.33	0.90	1.27	93.96	1.03	-5.15	71.33	0.79	-3.56	2.13	0.90	-0.003
GJB-2	6.54	1.00	-0.05	10.13	0.20	0.00	19.67	0.95	-0.62	68.76	1.03	-5.15	70.58	0.35	-3.57	2.23	0.97	-0.004
Green Long Cluster	16.81	1.00	-0.05	5.50	1.06	-0.01	20.33	1.20	8.90	107.03	1.03	-5.15	59.25	1.37	-3.73	1.93	0.90	-0.003
Gujarat Oblong brinjal-1	15.54	1.00	-0.05	12.63	1.06	-0.01	15.00	0.80	8.85	93.46	1.03	-5.15	60.25	1.37	-3.73	1.83	0.97	-0.004
IVBL-1	19.64	1.00	-0.05	4.97	1.06	-0.01	21.33	1.10	1.30	107.13	0.85	-5.10	71.17	1.48	-3.70	3.87	1.81	-0.002
IVBL-9	21.98	1.00	-0.05	5.83	1.06	-0.01	18.00	0.90	1.27	80.83	1.03	-5.15	69.33	0.79	-3.56	3.53	1.58	-0.004
JB-15	14.24	1.00	-0.05	5.27	1.06	-0.01	19.33	1.36	29.81	125.13	1.03	-5.15	70.75	0.53	-3.70	2.08	0.88	-0.004
MDU-1	8.14	1.00	-0.05	6.83	1.06	-0.01	9.67	0.69	21.49	56.06	1.03	-5.15	78.67	1.10	-3.58	2.00	0.99	-0.003
Nurki	12.08	1.00	-0.05	6.57	1.06	-0.01	18.00	1.20	8.90	67.26	1.03	-5.15	60.25	1.37	-3.73	1.93	0.82	-0.003
Pant Rituraj	9.89	1.21	0.09	9.55	0.57	-0.01	12.00	0.90	1.27	76.63	1.03	-5.15	70.42	0.53	-3.70	1.88	0.88	-0.004

Punjab Barsati	9.61	1.17	0.21	6.10	1.06	-0.01	15.33	1.00	-1.25	76.93	1.03	-5.15	69.17	0.26	-3.43	1.85	0.88	-0.004
Punjab Nagina	10.54	1.00	-0.05	5.60	1.06	-0.01	16.33	0.90	1.27	83.96	1.03	-5.15	69.50	0.93	-3.25	2.03	0.95	-0.003
Punjab Sadabahar	17.98	1.00	-0.05	3.67	1.06	-0.01	19.00	1.10	1.30	112.49	1.03	-5.15	69.58	1.37	-3.73	1.88	0.86	-0.003
Pusa Kranti	15.74	1.00	-0.05	6.83	1.06	-0.01	16.00	0.95	-0.62	78.63	1.03	-5.15	68.58	1.37	-3.73	2.04	1.05	-0.003
Pusa Shree	10.44	1.00	-0.05	4.73	1.06	-0.01	16.50	1.18	6.53	75.63	1.03	-5.15	58.25	1.37	-3.73	1.84	0.84	-0.004
DBR-31 (Pusa Uttam)	5.61	1.00	-0.05	4.87	1.06	-0.01	9.33	1.15	4.47	80.09	1.03	-5.15	70.25	0.35	-3.57	1.89	0.90	-0.003
Ramnagar Giant	13.94	1.00	-0.05	14.43	1.06	-0.01	6.50	0.72	17.86	94.89	1.03	-5.15	60.25	1.37	-3.73	2.23	0.93	-0.003
Swarna Mani	7.74	1.00	-0.05	7.60	1.06	-0.01	16.00	1.00	-1.25	84.93	1.03	-5.15	70.58	1.37	-3.73	2.70	1.36	-0.004
Swarna Prabha	11.08	0.75	0.02	4.73	1.06	-0.01	15.83	0.92	0.17	94.83	1.03	-5.15	70.75	0.99	-3.65	1.89	0.90	-0.003
Swarna Pratibha	16.91	1.00	-0.05	4.67	1.06	-0.01	17.67	1.15	4.47	85.73	1.03	-5.15	69.67	1.10	-3.58	1.94	1.05	-0.003
Swarna Shobha	7.80	0.78	0.07	8.50	1.06	-0.01	12.67	1.00	-1.25	75.69	1.03	-5.15	69.33	0.79	-3.56	1.79	0.74	-0.004
Swarna Shree	7.71	1.00	-0.05	6.47	1.06	-0.01	11.83	1.08	0.19	74.53	1.03	-5.15	77.58	1.37	-3.73	1.82	0.91	-0.003
Swarna Shyamli	11.78	1.00	-0.05	14.33	1.06	-0.01	11.17	0.82	6.48	77.16	1.03	-5.15	70.33	0.24	-3.56	2.08	1.09	-0.004
TRB-9	10.51	1.00	-0.05	6.73	1.06	-0.01	17.17	0.92	0.17	88.93	1.03	-5.15	71.33	0.79	-3.56	1.94	1.05	-0.003
Utkal Jyoti	15.71	1.00	-0.05	5.33	1.06	-0.01	19.50	0.98	-1.09	68.89	1.03	-5.15	71.40	1.25	-3.74	2.13	0.95	-0.003
Utkal Keshari	13.11	1.00	-0.05	6.50	1.06	-0.01	21.00	0.95	-0.62	90.73	1.03	-5.15	69.25	1.37	-3.73	1.96	0.95	-0.003
Utkal Madhuri	12.78	1.00	-0.05	4.77	1.06	-0.01	22.17	1.03	-1.09	89.19	1.03	-5.15	71.44	0.64	-3.42	2.08	1.01	-0.004
Utkal Tarini	7.84	1.00	-0.05	6.00	1.06	-0.01	21.67	1.00	-1.25	75.23	1.03	-5.15	61.92	1.37	-3.73	1.66	0.90	-0.003
Uttra	14.18	1.00	-0.05	3.97	1.06	-0.01	20.75	1.09	0.70	44.69	1.03	-5.15	70.42	0.12	-3.54	1.64	0.80	-0.003
PM ± SE	12.3 ± 0.07	1.0 ± 0.09		6.8 ± 0.02	1.0 ± 0.09		17.7 ± 1.45	1.0 ± 0.49		87.7 ± 0.06	1.0 ± 0.04		68.7 ± 0.20	1.0 ± 0.16		2.1 ± 0.01	1.0 ± 0.09	

m= mean; b= regression coefficient; S²di= deviation from regression; FL=Fruit length (cm); FD=Fruit diameter; NFPP=Number of fruit/plant; PH=Plant height; TPR=Time of physiological ripeness (days after fruit set); FYPP=Fruit yield/plant; PM±SE= Pooled mean± standard error.

The fruit size ratio was calculated by dividing the fruit length value by the fruit width value (L/W). In our finding of fruit length and diameter ratio, a cultivar PR-5 was small in fruit size, while, 39 (48.15%) and 41 (50.62%) cultivars were in medium and large fruit size, respectively (Table S3). Whereas, the general shape of fruits for all cultivars were categorized into globular (15), ovoid (22), obovate (11), pear-shaped (2), club-shaped (13), ellipsoid (4) and cylindrical (14). On the basis of the diameter of pistil scar the cultivars were classified into two groups of small (75=92.59%) and large (6=7.41%). In case of shape of apex, 31 (38.27%) cultivars showed indented, 11 (13.58%) were flattend, while, 19 (23.46%) and 20 (24.69%) cultivars had rounded and pointed shape of apex, respectively. The curvature in fruits was recorded only from 26 cultivars which were cylindrical type of fruits (Figure 4). Among the 26 cylindrical cultivars, the curvature was absent in 4, slight in 11 and medium in 10 cultivars, only a cultivar 'Swarna Abhilamb' exhibited strong curvature in their fruits (Table S3).

The fruit color of the skin at commercial harvesting was observed in three colors *i.e.* white, green, and purple with

their intensity of light, medium and dark colors (Figure 4). In the present study, only 5 (6.17%) cultivar's fruits were in white color, while, 18 cultivar's fruits were in green color and rest 58 cultivar's fruits exhibited purple color with different intensity of light, medium and dark (Table S3). The density of stripes was absent on fruits of 67 cultivars but the remaining 14 cultivars showed presence of the strips in density of spares (6), medium (6), and strong (2). Whereas, the paches on base of fruits were absent on 43 (53.09%) cultivars and present on 38 (46.91%) cultivars. In case of fruit glossiness at harvest maturity, all the 21, 38 and 22 cultivars showed week, medium and strong glossiness on fruits, respectively (Figure 4).

In the present study, the size of calyx on fruits were small (31 cultivars' 38.27%), medium (43 cultivars' 53.09%) and large (7 cultivars' 8.64%). In favor of calyx color and their intensity, a total of 71 cultivars had green color and their color intensity was in the range of weak (17 cultivars), medium (44 cultivars) and strong (10 cultivars). Remaining, 10 cultivars were in purple color with the color intensity of weak (4), medium (5) and strong (1), respectively (Table S3).

In case of spininess of calyx, the spines were not found on the calyx of 71 (87.65%) cultivar's but in 10 cultivars spines were present in different intensities of weak (5), medium (2) and strong (3). The ribs on fruits was absent in 63 (77.78%) cultivars while 18 (22.22%) cultivar's fruits showed presence of ribs in the intensity of weak (10), medium (6) and strong (2). Creasing of calyx on fruits displayed in weak (30 cultivars), medium (31 cultivars) and strong (20 cultivars) positions. The color of flesh in brinjal fruits was recorded into whitish and greenish colors of 18 (22.22%) and 63 (77.78%) cultivars, respectively. Whereas length of peduncle was expressed as medium in 45 (55.56%) and long in 36 (44.44%) cultivars, while, none was with small peduncle size (Table S3). In the present study it was also observed that the color of skin at physiological maturity of fruits changed to yellow (47' 59.26%' cultivars), orange (SB-1 and TRB-9) and brown (32' 38.27%' cultivars) colors (Table S4; Figure 4).

Feature of Plant Ideotype

The fruiting pattern of brinjal was solitary in 49 (60.49%) cultivars, cluster in 14 (17.28%) cultivars and rest of the 18 (22.22%) cultivars showed mixed fruiting pattern (Table S4). Growth habit of plants were classified into four categories e.g., erect (10 cultivars), semi-spreading (28 cultivars), spreading (38 cultivars) and horizontal (5 cultivars). Height of brinjal plants were recorded as short (30–60 cm) for 7 (8.64%) cultivars, medium (61–100 cm) for 46 (56.79%) cultivars and tall (101–150 cm) for 28 (34.57%) cultivars but none of the cultivar was measured as very short (<30 cm) plant height. However, the spreading nature of the plants were measured as narrow (<50 cm) in 5 cultivars, medium (50–100 cm) in 48 (58.02%) cultivars and broad (>100 cm) in 28 (35.80%) cultivars (Table S4). In context of timing of the physiological ripening of brinjal fruits, among the 81 cultivars only 29 (35.80%) expressed as early ripeness within 65 days after fruit set. Whereas, 15 (18.52%) cultivars showed medium ripeness between 65 to 75 days and rest 37 (46.68%) cultivars recorded as late ripeness on more than 75 days after fruit set (Table S4).

Genetic Diversity Analysis

In the present study, a high level of genetic diversity occurred (Figure 5). A dendrogram was constructed based on the pheno-morphometric traits of 81 extant cultivars of brinjal which segregated into ten (1-10) sub-cluster from four major clusters ('A', 'B', 'C', 'D'). A total of 31 extant cultivars were grouped in cluster 'A' by adding sub-cluster 1-3' with a coefficient range of 0.02–0.23%. The results showed that the cultivars' Arka Neelkanth', 'DBR-3' and 'Punjab Nagina' of sub-cluster '1' and the cultivars' Arka Sheel' and 'DBR-8' (Pusa Upkar) of sub-cluster '2' were close to each other. In another cluster 'B' which compiled of 18 extant cultivars from sub-cluster 4 and 5, out of which five cultivars' Arka Kusumakar', 'Arka Shree', 'KT-4 (Pusa Anupam)', 'PLR-1', and

Pusa Ankur' of sub-cluster '4' were displayed very close in coefficient range of 0.07–0.23%. However, cluster 'C' had amassed four sub-cluster (6-9) by including 30 extant cultivars of brinjal with coefficient range of 0.01–0.23%. Among thirty, the cultivars' Azad Brinjal-1' and 'IVBL-1 (Kashi Prakash)' of sub-cluster '6', the cultivars' Gujrat Oblong Brinjal-1' and 'Ramnagar Giant' of sub-cluster '7', and the cultivars' Azad Brinjal-2', 'CHBR-2' and 'MDU-1' of sub-cluster '9' displayed closeness with each other. While, the cluster 'D' was separated along with sub-cluster '10', including two cultivars, 'DBL-329' and 'Swarna Shree' and they were found to be close with each other in a coefficient range of 0.07–0.23%.

Stability Test Analysis

The stability test analysis exposed that the estimates of variances of variety, environment, environment $\times$ variation $\times$ environment and environment (linner) were highly significant for all characters (Table 1). The estimates of mean (m), regression coefficient (b) and deviation from regression (s^2_{di}) for yield related characters (fruit length, fruit diameter, number of fruit/plant, plant height, time of physiological ripeness and fruit yield/plant) were calculated for stability test between 48 extant cultivars of brinjal (Table 2). In results of the stability test of fruit length, all the cultivars showed equal to '1' ($b=1$) average response of regression coefficient and -0.05 deviation from regression excluding four cultivars wherein 'Pant Rituraj' and 'Punjab Barsati' had more than '1' ($b>1$) and the cultivars' Swarna Pratibha' and 'Swarna Shobha' showed less than '1' ($b<1$) average response of regression coefficient. While, for the characters fruit diameter all the 45 extant cultivars had equal to '1' ($b=1$) average response of regression coefficient and -0.01 deviation from regression but three cultivars' Arka Nidhi', 'GJB-2' and 'Pant Rituraj' showed less than one ($b<1$) average response of regression coefficient. For the trait number of

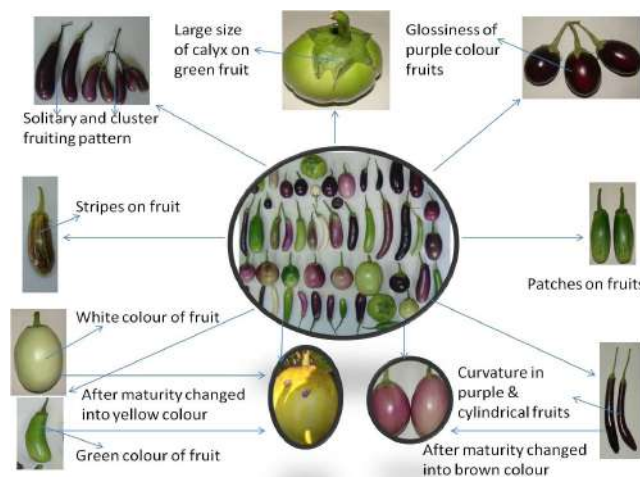


Figure 4: Diversity, size of calyx, strip and patches, glossiness, fruiting pattern, curvature, maturity stages of the fruits of brinjal.

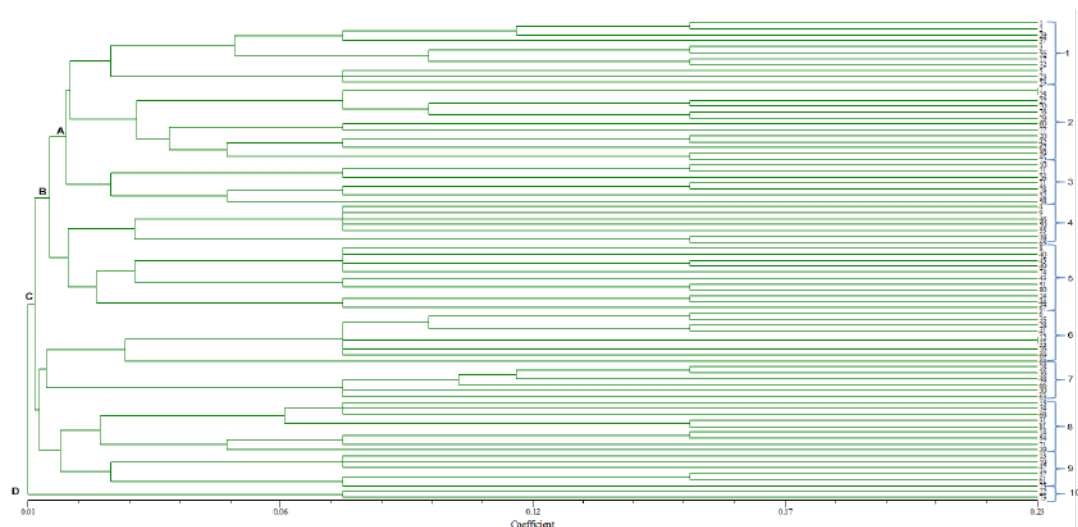


Figure 5: Unweighted Pair Group Method with Arithmetic mean (UPGMA) dendrogram (based on Euclidean distance coefficient) of 81 extant cultivars of brinjal generated by their morphometric traits. [1-81 extant cultivar's code stand for: 1=Annamali; 2=ArkaKeshav; 3=ArkaKranti; 4=ArkaKusumakar; 5=ArkaNeelkanth; 6=ArkaNidhi; 7=ArkaSheel; 8=ArkaShirish; 9=Arka Shree; 10=Aruna; 11=Ausrey; 12=Azad Brinjal-1; 13=Azad Brinjal-2; 14=Azad Brinjal-3; 15=Azad Brinjal-4; 16=Bhagyamati; 17=BR-14; 18=CH-1045; 19=CHBR-2; 20=CO-1; 21=CO-2; 22=DBL-329; 23=DBR-3; 24=DBR-31 (PusaUttam); 25=DBR-8 (PusaUpkar); 26=DRNKV-2-29; 27=GBL-1; 28=GJB-2; 29=Green Long Cluster; 30=Gujrat Oblong Brinjal-1; 31=Gulabi; 32=IBL-116-135; 33=IVBL-1 (Kashi Prakash); 34=IVBL-10; 35=IVBL-9 (KashiTaru); 36=JB-15; 37=JB-6; 38=JB-65; 39=JB-67; 40=JB-80; 41=JBGR-1; 42=JBL-03-04; 43=JBL-116-113; 44=KKM-1; 45=KS-224; 46=KT-4 (PusaAnupam); 47=MDU-1; 48=Nurki; 49=Pant Rituraj; 50=PLR-1; 51=PR-5; 52=Punjab Barsati; 53=Punjab Nagina; 54=Punjab Sadabahar; 55=PusaAnkur; 56=PusaBindu; 57=PusaKranti; 58=Pusa Purple Cluster; 59=Pusa Purple Long; 60=Pusa Shree; 61=PusaShyamla; 62=Rajendra Brinjal-2; 63=Ramnagar Giant; 64=RCMBL-1; 65=SB-1; 66=Surya; 67=SwarnaAbhilamb; 68=Swarna Ajay; 69=Swarna Mani; 70=SwarnaPrabha; 71=SwarnaPratibha; 72=SwarnaShobha; 73=Swarna Shree; 74=SwarnaShyamali; 75=Swetha; 76=TRB-9; 77=UtkalJyoti; 78=UtkalKeshari; 79=UtkalMadhuri; 80=UtkalTarini; 81=Uttara].

fruits per plant the 24 cultivars showed less than one ($b < 1$), 8 cultivars equal to '1' ($b = 1$) and 16 cultivars more than one ($b > 1$) regression coefficient. Whereas, for the characters plant height 43 cultivars showed equal to one ($b = 1$) and 5 cultivars were having less than one ($b < 1$) regression coefficient. For the traits time of physiological ripening the 21 and 26 cultivar were found less than one ($b < 1$) and more than one ($b > 1$) regression coefficient but 'Arka Nidhi' had equal to one regression coefficient. Similarly, for the character fruit yield per plant, the 32, 8 and 8 cultivars exhibited less than one ($b > 1$), equal to one ($b = 1$) and more than one ($b < 1$) regression coefficient and close to '0' deviation from regression ($s^2 di = 0$) for all the cultivars (Table 2).

Discussion

In the results of the present study, the anthocyanin coloration was present on their hypocotyls at seedling stage and on the stem at vegetative stage in most of cultivars with different color intensity of weak, medium and strong. It has been reported that the anthocyanins are natural pigments found in plant parts including leaves and brinjal stem in various colors like pink, red, magenta, purple and dark blue (Chaudhary and Mukhopadhyay, 2012). The presence of anthocyanin in plants protected from the damage by UV radiations. It was also observed that the antioxidant ability is found in anthocyanins which have various health benefits for human and animal and also utilized by the pharmaceutical industries (Lila, 2004; Chalabi

et al., 2008; Vyas *et al.*, 2009; Gurbuz *et al.*, 2018). Similarly, pubescence (hairs) on the plant parts in different intensity of weak, medium and strong were found in many cultivars. Botanically these plant hairs are also called trichomes and have played major role in plant life cycle for example the dense coating of hair is protecting the slight growing parts and the living surface cells from cold and strong sunlight in open habitats. In windy locations, the hairs can also break up the flow of air across the plant surface, thereby reducing the transpiration rate in plant.

In this study, the leaf of all the cultivars exhibited different size, shape and nature like small, medium, large in size, leaf margin, presence of blistering and the intensity of spininess etc. Leaf shape and size is highly responsible for photosynthetic process to make food for the plants. Leaf spininess is helpful during transpiration as well as it protects the crop from birds and animals. Mostly wild species and their derivatives of brinjal bear spine on the leaves and stem, which indicated to resistance against insect and pest (Knapp *et al.*, 2013). Dash *et al.* (2019); Oladosu *et al.* (2021) and Younas *et al.* (2022) used the characteristics and importance of spininess in brinjal in their studies. It was also observed that the leaf blade and leaf vein color of all cultivars expressed green and purple in different intensity of light, medium and dark. These, green and purple color of leaf blade and leaf vein (Figure 3) was due to the presence of chlorophyll and anthocyanin color pigments. On the basis of phyto-physiological theory, these chlorophyll and anthocyanin color pigments are responsible for facilitating photosynthesis

in plants and for securing UV radiations (Chaudhary and Mukhopadhyay, 2012).

In case of number of flowers in inflorescence, 62.96% cultivars expressed 1 to 3 number of flowers and 37.04% cultivars were having more than three flowers in their each inflorescence. More number of flowers are directly related to more clusters which produce more number of fruits. Similarly, in case of flower size they displayed in small, medium and large size (Figure 4). It has already been reported that the large flower size containing big reproductive organs and pistil length that were made easy and high pollen absorption capacity during pollination because the nature of flowers of eggplant showed heterostyly phenomenon (Oladosu *et al.*, 2021; Kowalska, 2006). Whereas, the pistil length in flowers of eggplant depends on the plant's age (Kowalska, 2008). In response of our study it has been clarified that mostly eggplant produced maximum fruits from high-pistil flower in range of 49–100%, medium fruit's from medium-pistil flowers in the range of 46–85% and less fruit's from low-pistil flowers in the range of 10–20% (Younas *et al.*, 2022; Kowalska, 2003, 2006, 2008). The flower color was greenish white, light purple, purple and dark purple among the cultivars which may be due to the presence of xanthophyll color pigments. The present results corroborate the findings of Polignano *et al.* (2010). It was also reported that the flowering times in cultivars were classified into three categories early, medium and late from days after seed sowing. Rai *et al.* (2005) has studied that earliness in flowering of any crop is influencing the duration of early fruiting and maturity.

In this study, fruit size was measured as small, medium, large and different shapes such as globular, ovoid, obovate, pear-shaped, club-shaped, ellipsoid and cylindrical. Fruit size and shape are most important characters in brinjal for deciding their demand in markets and commercialization but it depends on the places and interest of people (Adeniji *et al.*, 2013). For example, long and small fruited varieties have been demanded in tropical India and China. Prominent size and shape of brinjal (Figure 5) have been selected by breeders in their breeding program for improving or developing new varieties (Adeniji *et al.*, 2013). Another measurement- for diameter of pistil scar, shape of apex, and curvature in fruits were recorded among the cultivars. Practically we know that the diameter of pistil scar, shape of apex and curvature in fruits are also responsible for deciding the quality of brinjal fruits. Similar justification has been given by Adeniji and Aloyce (2012). The absence and presence of strips, patches and glossiness on fruits were also recorded during the study. As we had assume that the high density of strips, presence of more patches and low glossiness of fruits are affected to the markets of brinjal because in our view the fresh and good looking fruits are to be popular in plain region population. At commercial harvesting, the color of brinjal fruits was white, green, and purple with light, medium and dark intensity. In this context, it has been reported that the color of brinjal fruits expressed is due to

the presence of anthocyanins and chlorophyll pigments. These pigments are found naturally in plant parts and fruits in different colors (green, pink, red, magenta, purple and dark blue) and depends on the pH effects (Polignano *et al.*, 2010; Chaudhary and Mukhopadhyay, 2012). However, white brinjal fruits indicated these color pigments' absent or poor presence. The light, medium and dark green colors on fruits peel indicated presence of chlorophyll color pigments and may be helpful during the photosynthetic process. Whereas the light, medium and dark purple peel colors of fruits indicated the presence of significant amount of phenolic flavonoid phytochemicals. Previously, a number of scientific and epidemiological reports suggested that those vegetables containing anthocyanins or anthocyanin extracts will be beneficial for human and animal health against cancer, aging, inflammation and neurological diseases due to presence of antioxidant ability and also utilized by pharmaceutical industries (Chalabi *et al.*, 2008; Vyas *et al.*, 2009; Chaudhary and Mukhopadhyay, 2012).

In the present study, many characters on brinjal fruits like size of calyx, color of calyx, spininess of calyx, creasing of calyx, color of flesh in fruits, length of peduncle were recorded. In case of less size of calyx, more spininess on calyx, high creasing of calyx, short peduncle size may reduce the market value of brinjal fruits. Less size of calyx and peduncle of flower may indicate a typical symptom of the particular varieties (Younas *et al.*, 2022; Kowalska, 2006). The spininess, deep color, more creasing on calyx and greenish color of fruits are used by South Indian or African people because these people choose such type of brinjal for preparing delicious dishes (Oladosu *et al.*, 2021; Kouassi *et al.*, 2014). Further in this study it resulted that the color of the skin at physiological maturity of fruits changed into yellow, orange and brown colors among the cultivars. The changes in color is an indication of physiological maturity of fruits and that it is ready to be harvested for seed. It was also observed that those cultivar's fruits which were white and green in color at commercial stage turned into yellow and orange color at the maturity. However, those cultivar's having purple color at commercial stage changed into brown color at maturity (Table S4; Figure 4). Earlier it has been studied that during the maturity of fruits, decoloration or degradation starts in anthocyanin or chlorophyll pigment due to secretion of ethylene (Kader, 1996). They observed that the chlorophyll (green color) was lost gradually during fruit maturity and the carotenoids (red, yellow and orange color) increases and induces yellowing of green tissues.

In present study, many characters like fruiting pattern (solitary and mixed), growth habit of plants (erect, semi spreading, spreading and horizontal), plants height (very short, short, medium and tall), the spread nature of plants (narrow, medium and broad) was observed under plant ideotypes. The fruiting pattern depends on the number of flower in an inflorescence, whereas, vigorousness in height, spreading nature, and growth habit of plants are representing

the behaviour of varieties and may be responsible for producing high yield (Figure 4). Present finding is similar to earlier reports of Hazra *et al.* (2003) and Kouassi *et al.* (2014). Regarding physiological ripeness, 35.80, 18.52 and 46.68% cultivars were exhibited early, medium and late, respectively. Early or medium days of maturity and ripening of fruits may be good for packaging and commercialization of seed by entrepreneurs and companies and also will be suitable for getting early cultivation of crop in future (Kouassi *et al.*, 2014; Oladosu *et al.*, 2021; Younas *et al.*, 2022).

In results of genetic diversity, four major clusters ('A', 'B', 'C' and 'D') have been constructed from 10 sub-clusters by including 81 extant cultivars with a coefficient range of 0.01 to 0.23%. Several cultivars from each cluster indicated genetically close relationships with each other. This study of genetic relatedness is agreed by (Karak *et al.*, 2012; Solaiman *et al.*, 2014). Whereas, clusters 'A' and 'C' had high genetic divergence because of different genetic morphology. Genetic divergence in brinjal was studied by Karak *et al.* (2012), Caguiat and Hautea (2014) and Solaiman *et al.* (2014) for morphological traits. The present study indicated that the brinjal cultivars having high genetic diversity may be helpful for selection of trait-specific parent in breeding program. In present study, the cultivars 'Arka Kusumakar', 'Arka Shree', 'KT-4 (Pusa Anupam)', 'PLR-1', and 'Pusa Ankur' of cluster 'B' and sub-cluster '4' displayed closeness. While, two cultivars 'Uttara' of Bangladesh and 'Nurki' of Nepal grouped in cluster 'C' of sub-cluster '7' and '8'. These cultivars of cluster 'B' and 'C' adopted similar phenetic traits of indigenous cultivars or may be developed with similar genetic backgrounds by indigenous cultivars. They have accepted the similar morphology, it may be due to the maintenance and conservation of these cultivars in the same environment. It also resulted that only two cultivars ('DBL-329' and 'Swarna Shree') were grouped with cluster 'D' and sub-cluster '10'; these cultivars may have different morphology. Earlier, it has been studied that the successful conservation of gene pools is largely dependent on environmental interaction, stability and understanding the diversity (Solaiman *et al.*, 2014; Caguiat and Hautea 2014).

In stability test analysis the variances of variety, environment, environment + variation $\times$ environment and environment (line) were highly significant for all characters. It means the cultivars were stable in each morphological character environment (Eberhart and Russell, 1966). In results of the stability test of fruit length all the cultivars showed equal to '1' ($b=1$) average response of regression coefficient and -0.05 deviation from regression but the cultivars 'Swarna Pratibha' and 'Swarna Shobha' were less than '1' ($b<1$) regression coefficient. While, in case of fruit diameter 45 extant cultivars had equal to '1' ($b=1$) and three cultivars, 'Arka Nidhi', 'GJB-2' and 'Pant Rituraj' were less than one ($b<1$) regression coefficient and -0.01 deviation from regression. According to Eberhart and Russell (1966), the $b<1$ and $b=1$ regression coefficient along with $S^2di=0$

deviation from regression indicated the stability for these cultivars against fruit length and diameter. For the character number of fruit per plant, 24 and 8 cultivars showed $b<1$ and $b=1$ regression coefficient, for plant height five cultivars showed less than '1' and 43 cultivars were showed equal to '1' regression coefficient. These stable cultivars can be cultivated for these traits in any environment. It was also observed that the trait time of physiological ripening, 21 cultivars exhibited less than one ($b<1$) and 'Arka Nidhi' had equal to one regression coefficient. These 22 stable cultivars can be cultivated for earliness in any climatic region. Correspondingly, for the character fruit yield per plant 32 and 8 cultivars were exhibited less than one ($b>1$) and equal to one ($b=1$) regression coefficient and also displayed close to '0' deviation from regression ($S^2di=0$). Therefore, these cultivars can be cultivated friendly and utilized during the breeding programme for improving yield capacity in any climatic zone. In support of this finding several workers has been reported that the results of low or equal regression coefficient along with zero deviation from regression indicated most stable and adaptive nature of crops (Eberhart and Russell, 1966; Mehta *et al.*, 2011; Sivakumar *et al.*, 2015; Bhushan and Samnotra, 2017).

Conclusion

In the present study, it was concluded that all 81 extant cultivars of brinjal exhibited distinctiveness and uniformity on the basis of 47 morphometric characters. Each character displayed their economic importance for example, purple color in seedling, stem, leaves, flowers and fruits is due to the high intensity of anthocyanin color pigmentation and protects to plant from UV rays; the presence of hairs and spine on leaf, calyx and stem protects plant from frost, speedy wind and insect pest attack. The size, shape and color of fruits may be helpful during their marketing. The plant vigour ideotypes and earliness in fruiting may be helpful for improving the yield, etc. Moreover, the characterization of these cultivars on the basis of their various morphological traits would be helpful for generating a database on brinjal, and also will be helpful for researchers and students to know about brinjal from a single platform. The assessment of the genetic diversity and relationships between the brinjal cultivars could be utilized in conservation, breeding and crop evolution. Thus the stable cultivars can be grown in any environment and could be utilized in the varietal improvement programme of brinjal for desired characteristics. Furthermore, these cultivars can be protected and registered under PPV&FRA, New Delhi on the basis of DUS testing.

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Table S1 (Suppl.): Serial/code number*, name and pedigree/parentage of 81 extant cultivars of brinjal and their origin centre

Serial/Code number*	Name of extant cultivars	Pedigree (parentage) of extant cultivars	Origin centre
1	Annamali	Selection from AC-49 A germplasm	Tamil Nadu Agricultural University (TNAU), Coimbatore, India
2	Arka Keshav	Dingrass Multiple Purple x Arka Sheel	ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta lake post, Bangalore, Karnataka, India
3	Arka Kranti	Pure line selection	ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta lake post, Bangalore, Karnataka, India
4	Arka Kusumakar	Pure line selection from IIHR 193	ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta lake post, Bangalore, Karnataka, India
5	Arka Neelkanth	Dingrass Multiple Purple x Arka Sheel (BWR- 54).	ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta lake post, Bangalore, Karnataka, India
6	Arka Nidhi	Dingrass Multiple Purple x Arka Sheel (BWR-12)	ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta lake post, Bangalore, Karnataka, India
7	Arka Sheel	IIHR 192 (a local collection from Kodagu, Karnataka)	ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta lake post, Bangalore, Karnataka, India
8	Arka Shirish	Pure line selection from IIHR 194-1 (local collection of Karnataka)	ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta lake post, Bangalore, Karnataka, India
9	Arka Shree	Pure line selection	ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta lake post, Bangalore, Karnataka, India
10	Aruna	Selection from Local cultivars	Dr. Panjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola, Maharashtra
11	Ausrey	Pure line selection	Kerala Agricultural University (KAU), Vellanikkara, Thrissur, Kerala, India
12	Azad Brinjal-1	Banarasi Giant Round x 7103	Chandra Shekhar Azad University of Agriculture and Technology (CSAU&T), Kanpur, Uttar Pradesh, India
13	Azad Brinjal-2	Pusa Purple Round x Arka Sheel	Chandra Shekhar Azad University of Agriculture and Technology (CSAU&T), Kanpur, Uttar Pradesh, India
14	Azad Brinjal-3	Selection	Chandra Shekhar Azad University of Agriculture and Technology (CSAU&T), Kanpur, Uttar Pradesh, India
15	Azad Brinjal-4	Selection	Chandra Shekhar Azad University of Agriculture and Technology (CSAU&T), Kanpur, Uttar Pradesh, India
16	Bhagyamati	Selection from Local cultivars	Acharya N. G. Ranga Agricultural University (ANGRAU), Hyderabad, Telangana
17	BR-14	Selection	ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi, Uttar Pradesh, India
18	CH-1045	Selection	ICAR-Horticulture and Agro-forestry Research Programme (HARP), Ranchi, Jharkhand, India
19	CHBR-2	Selection	ICAR- Central Horticultural Experiment Station (CHES), Bhubaneswar
20	CO-1	Pureline selection	Tamil Nadu Agricultural University (TNAU), Coimbatore, India
21	CO-2	Selection by local variety 'Varikkathiri' of Negamum, Coimbatore	Tamil Nadu Agricultural University (TNAU), Coimbatore, India
22	DBL-329	Selection	ICAR-Indian Agricultural Research Institute (IARI), Hill Side Road, Pusa, New Delhi, India
23	DBR-3	Selection	ICAR-Indian Agricultural Research Institute (IARI), Hill Side Road, Pusa, New Delhi, India
24	DBR-31 (Pusa Uttam)	GR x Pant Rituraj	ICAR-Indian Agricultural Research Institute (IARI), Hill Side Road, Pusa, New Delhi, India
25	DBR-8 (Pusa Upkar)	GR x PB 91-1	ICAR-Indian Agricultural Research Institute (IARI), Hill Side Road, Pusa, New Delhi, India
26	DRNKV-2-29	Selection	ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi, Uttar Pradesh, India

27	GBL-1	Selection	Anand Agricultural University (AAU), Anand, Gujarat, India
28	GJB-2	Selection	Anand Agricultural University (AAU), Anand, Gujarat, India
29	Green Long Cluster	Selection	ICAR-Indian Institute of Horticultural Research (IIHR), Hessaraghatta lake post, Bangalore, Karnataka, India
30	Gujrat Oblong Brinjal-1	Selection	Anand Agricultural University (AAU), Anand, Gujarat, India
31	Gulabi	Selection	Acharya N. G. Ranga Agricultural University (ANGRAU), Hyderabad, Telangana
32	IBL-116-135	Pure line selection	ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi, Uttar Pradesh, India
33	IVBL-1 (Kashi Prakash)	Selection	ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi, Uttar Pradesh, India
34	IVBL-10	Pure line selection	ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi, Uttar Pradesh, India
35	IVBL-9 (Kashi Taru)	Mass selection	ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi, Uttar Pradesh, India
36	JB-15	Pure line selection	Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh
37	JB-6	Pure line selection	Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh
38	JB-65	Pure line selection	Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh
39	JB-67	Pure line selection	Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh
40	JB-80	Pure line selection	Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh
41	JBGR-1	Pure line selection	Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh
42	JBL-03-04	Pure line selection	Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh
43	JBL-116-113	Pure line selection	Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh
44	KKM-1	Pure line selection from Kulathur local near Tirunelveli	Tamil Nadu Agricultural University (TNAU), Coimbatore, India
45	KS-224	Selection	Chandra Shekhar Azad University of Agriculture and Technology (CSAU&T), Kanpur, Uttar Pradesh, India
46	KT-4 (Pusa Anupam)	PPC x Pusa Kranti	ICAR-Indian Agricultural Research Institute (IARI), Regional Station, Katrain, New Delhi, India
47	MDU-1	Selection from Kallampati local type near Madurai	Tamil Nadu Agricultural University (TNAU), Coimbatore, India
48	Nurki	Introduction	Nepal Agricultural Research Council (NARC), Nepal
49	Pant Rituraj	Type-3 x Pusa Purple Cluster	Govind Ballabh Pant University of Agriculture and Technology (GBPUA&T), Pantnagar, Uttarakhand, India
50	PLR-1	Reselection from a Nagpur ecotype	Tamil Nadu Agricultural University (TNAU), Coimbatore, India
51	PR-5	Selection	ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi, Uttar Pradesh, India
52	Punjab Barsati	PPC x PH-4	Punjab Agricultural University (PAU), Ludhiana, Punjab, India
53	Punjab Nagina	Pure line selection	Punjab Agricultural University (PAU), Ludhiana, Punjab, India
54	Punjab Sadabahar	PPC x H-4	Punjab Agricultural University (PAU), Ludhiana, Punjab, India
55	Pusa Ankur	Progeny selection of GR x 91-1	ICAR-Indian Agricultural Research Institute (IARI), Hill Side Road, Pusa, New Delhi, India

Table continued....

56	Pusa Bindu	GR x Pant Rituraj	ICAR-Indian Agricultural Research Institute (IARI), Hill Side Road, Pusa, New Delhi, India
57	Pusa Kranti	[(PPL x Hyderpur)x Wynad Giant] three way	ICAR-Indian Agricultural Research Institute (IARI), Hill Side Road, Pusa, New Delhi, India
58	Pusa Purple Cluster	Selection from local material	ICAR-Indian Agricultural Research Institute (IARI), Hill Side Road, Pusa, New Delhi, India
59	Pusa Purple Long	Selection from Bhatiya cultivar	ICAR-Indian Agricultural Research Institute (IARI), Hill Side Road, Pusa, New Delhi, India
60	Pusa Shree	Selection	ICAR-Indian Agricultural Research Institute (IARI), Hill Side Road, Pusa, New Delhi, India
61	Pusa Shyamla	Sel NDB-25-11 x Sel HE-12-2	ICAR-Indian Agricultural Research Institute (IARI), Hill Side Road, Pusa, New Delhi, India
62	Rajendra Brinjal-2	Selection	Dr. Rajendra Prasad Central Agriculture University (RPCAU), Samastipur, Bihar
63	Ramnagar Giant	Selection	ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi, Uttar Pradesh, India
64	RCMBL-1	Selection	ICAR-Research Complex NEH region, Barapani, Meghalaya, India
65	SB-1	Selection	ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi, Uttar Pradesh, India
66	Surya	SM6-7 (SPS)	Kerala Agricultural University (KAU), Vellanikkara, Thrissur, Kerala, India
67	Swarna Abhilamb	Pure line selection from germplasm of Dubani Dish, Assam	ICAR-Horticulture and Agro-forestry Research Programme (HARP), Ranchi, Jharkhand, India
68	Swarna Ajay	Pureline Selection	ICAR-Horticulture and Agro-forestry Research Programme (HARP), Ranchi, Jharkhand, India
69	Swarna Mani	Selection	ICAR-Horticulture and Agro-forestry Research Programme (HARP), Ranchi, Jharkhand, India
70	Swarna Prabha	Selection	ICAR-Horticulture and Agro-forestry Research Programme (HARP), Ranchi, Jharkhand, India
71	Swarna Pratibha	Pureline Selection	ICAR-Horticulture and Agro-forestry Research Programme (HARP), Ranchi, Jharkhand, India
72	Swarna Shobha	Pure line selection	ICAR-Horticulture and Agro-forestry Research Programme (HARP), Ranchi, Jharkhand, India
73	Swarna Shree	Pureline Selection	ICAR-Horticulture and Agro-forestry Research Programme (HARP), Ranchi, Jharkhand, India
74	Swarna Shyamali	Pure line selection	ICAR-Horticulture and Agro-forestry Research Programme (HARP), Ranchi, Jharkhand, India
75	Swetha	SM6-6 (SPS)	Kerala Agricultural University (KAU), Vellanikkara, Thrissur, Kerala, India
76	TRB-9	Selection	Tamil Nadu Agricultural University (TNAU), Coimbatore, India
77	Utkal Jyoti	KT-4 x BB-11	Orissa University of Agriculture and Technology (OUA&T), Bhubaneswar, Odisha, India
78	Utkal Keshari	BB-11 x KJ-3-1	Orissa University of Agriculture and Technology (OUA&T), Bhubaneswar, Odisha, India
79	Utkal Madhuri	PBR-125-5 x Pipili-4	Orissa University of Agriculture and Technology (OUA&T), Bhubaneswar, Odisha, India
80	Utkal Tarini	Pusa Kranti x Gopa Local	Orissa University of Agriculture and Technology (OUA&T), Bhubaneswar, Odisha, India
81	Uttara	Introduction	Bangladesh Agricultural Research Institute (BARI), Bangladesh

*Code number used for name of cultivars in Table S2, S3, S4.

Table S2 (Suppl.): Morphological characteristic of seedling stems and leaves in 81 extant cultivars of brinjal

<i>Plant parts: Characters-serial numbers (type of assessment/stage of observation)</i>	<i>States (Observation note)</i>	<i>No. of cultivars</i>	<i>Per cent of cultivars</i>	<i>Cultivars code no.*</i>
Seedling: intensity of anthocyanin coloration of hypocotyl - 1-2 (VG & VS)	Absent (1)	23	29.63	4, 7-10, 12, 20, 22, 23, 25-27, 29, 31, 35, 41, 44, 48, 51, 60, 61, 63, 70
	Weak (3)	18	20.99	2, 5, 14, 17, 21, 24, 33, 36, 37, 38, 46, 47, 54, 55, 62, 64, 65, 76
	Medium (5)	20	24.69	3, 6, 11, 13, 16, 18, 30, 34, 39, 40, 43, 45, 50, 52, 53, 58, 59, 72, 75, 78
	Strong (7)	17	20.99	1, 15, 19, 28, 32, 42, 49, 66, 68, 69, 71, 73, 74, 77, 79, 80, 81
	Very strong (9)	3	3.70	56, 57, 67
Stem: intensity of anthocyanin coloration-3-4 (VG & VS)	Absent (1)	35	43.21	4, 7-10, 12, 14, 18, 20, 21, 23, 24, 26, 29, 32, 34, 40-45, 48, 49, 52-54, 59, 62-66, 70, 71
	Weak (3)	22	25.93	3, 5, 11, 15, 17, 25, 28, 31, 33, 36, 38, 46, 50, 51, 55-58, 60, 76, 79, 81
	Medium (5)	17	20.99	2, 6, 13, 16, 19, 22, 27, 30, 35, 37, 39, 47, 61, 69, 73-75,
	Strong (7)	2	2.47	77, 78
	Very strong (9)	5	7.41	1, 67, 68, 72, 80
Stem: pubescence-5 (VG)	Weak (3)	30	37.04	2, 3, 5, 9-11, 14-16, 19, 20, 25, 32, 34-36, 38, 39, 45, 49-52, 56, 57, 61, 63, 69, 75, 78
	Medium (5)	38	46.91	6-8, 12, 17, 18, 21, 23, 26, 27, 29-31, 33, 37, 40, 42-44, 46-48, 53, 55, 58-60, 62, 64, 65, 68, 70, 74, 76, 77, 79-81
	Strong (7)	13	16.05	1, 4, 13, 22, 24, 28, 41, 54, 66, 67, 71-73
Leaf: size area index-6-7 (length/width) (MS)	Small <1.0 cm (3)	0	0.00	0
	Medium 1.0–2.0 cm (5)	76	93.83	1, 2, 4-7, 9-29, 31-62, 64-76, 78-81
	Large >2.0 cm (7)	5	6.17	3, 8, 30, 63, 77
Leaf: margin-8 (VS)	Entire (1)	30	37.04	1, 4, 5, 10, 12-14, 16, 19, 20, 28, 33, 35-38, 42, 45-47, 52, 56, 62, 63, 65, 67, 70, 71, 73, 79,
	Dentate (3)	37	45.68	2, 6, 8, 9, 11, 15, 17, 18, 21, 24, 26, 27, 29, 30-32, 34, 39, 41, 44, 48-51, 57, 61, 64, 66, 68, 69, 72, 74-78, 80
	Sinuate (5)	14	17.28	3, 7, 22, 23, 25, 40, 43, 53-55, 58-60, 81
Leaf: blistering-9 (VG)	Absent (1)	35	43.21	1, 3, 6, 8, 10, 14, 16, 17, 20, 22, 23, 30, 31, 34-36, 43-45, 50, 53, 56, 57, 59, 61, 64, 68, 69, 71, 75-78, 80, 81
	Present (9)	46	56.79	2, 4, 5, 7, 9, 11-13, 15, 18, 19, 21, 24-29, 32, 33, 37-42, 46-49, 51, 52, 54, 55, 58, 60, 62, 63, 65-67, 70, 72-74, 79
Leaf: spininess and intensity-10-11 (VG & MG)	Absent (1)	75	92.59	1-40, 43-66, 69-71, 73, 75-81
	Weak <5 (3)	2	2.47	41, 42
	Medium 5–10 (5)	1	1.23	67
	Strong >10 (7)	3	3.70	68, 72, 74
Leaf: blade color and their intensity- 12-14 (VG)	Green (1) Light (3)	24	29.63	3, 5, 8, 11, 13, 14, 16-18, 20-23, 29, 41, 45, 46, 49, 55, 58, 60, 75, 79, 81
	Medium (5)	32	39.51	2, 4, 7, 9, 10, 12, 15, 24, 26, 31-35, 37-40, 42, 44, 51-53, 57, 59, 62-66, 70, 74
	Dark (7)	4	4.94	36, 43, 54, 76
	Purple (2) Light (3)	7	8.64	6, 19, 25, 27, 48, 69, 73,
	Medium (5)	8	9.88	28, 30, 47, 50, 61, 71, 72, 78
	Dark (7)	6	7.41	1, 56, 67, 68, 77, 80

<i>Plant parts: Characters-serial numbers (type of assessment/stage of observation)</i>	<i>States (Observation note)</i>		<i>No. of cultivars</i>	<i>Per cent of cultivars</i>	<i>Cultivars code no.*</i>
Leaf: color of vein and their intensity-15-16 (VG)	Green (1)	Light (3)	5	6.17	8, 20, 21, 43, 65
		Medium (5)	4	4.94	29, 44, 53, 79
		Dark (7)	1	1.23	4
	Purple (2)	Light (3)	30	37.04	2, 7, 9, 10, 13, 14, 17, 18, 22-24, 31-36, 38, 40-42, 46, 48, 54, 58-60, 62, 66, 75
		Medium (5)	21	25.93	3, 5, 11, 12, 15, 19, 26, 28, 30, 39, 45, 47, 50, 51, 55, 57, 63, 64, 70, 71, 76
		Dark (7)	20	24.69	1, 6, 16, 25, 27, 37, 49, 52, 56, 61, 67-69, 72-74, 77, 78, 80, 81

*Name and codes of cultivars are given in supplementary Table S1 (Supp.).

Table S3 (Suppl.): Morphological characteristics of flowers and fruits in 81 extant cultivars of brinjal

<i>Plant parts: Characters-serial numbers (type of assessment/stage of observation)</i>	<i>States (Observation note)</i>	<i>No. of cultivars</i>	<i>Per cent of cultivars</i>	<i>Cultivars code no.*</i>
Inflorescence: Number of flowers-17 (VG)	1 to 3 (1)	51	62.96	3-5, 7-10, 12-14, 17-20, 24, 28, 30-33, 35-39, 41, 42, 45, 46, 48-57, 59-61, 63, 64, 66, 67, 69-71, 73, 75
	>3 (2)	30	37.04	1, 2, 6, 11, 15, 16, 21-23, 25-27, 29, 34, 40, 43, 44, 47, 58, 62, 65, 68, 72, 74, 76-81
Flower: size- 18 (VG)	Small (3)	22	27.16	2, 4, 10, 11, 13, 16, 18, 21, 25, 26, 30, 34, 36, 44, 50-52, 56, 59, 66, 71, 81
	Medium (5)	36	44.44	1, 5-7, 12, 15, 20, 22, 23, 27, 29, 31, 35, 37-40, 42, 43, 45-49, 53, 55, 58, 68-70, 74, 75, 77-80
	Large (7)	23	28.40	3, 8, 9, 14, 17, 19, 24, 28, 32, 33, 41, 54, 57, 60-65, 67, 72, 73, 76
Flower: color- 19 (VS)	Greenish white (1)	4	4.94	8, 9, 29, 70
	Light purple (2)	19	23.46	2, 4, 10-12, 14, 18, 20, 21, 24, 25, 32, 41, 45, 51, 52, 60, 65, 76
	Purple (3)	39	48.15	3, 5, 7, 13, 15, 17, 22, 23, 26-28, 30, 31, 33-35, 37, 40, 42, 43, 46, 47, 49, 53-55, 58, 59, 61-64, 66, 69, 71, 73-75, 79
	Dark purple (4)	19	23.46	1, 6, 16, 19, 36, 38, 39, 44, 48, 50, 56, 57, 67, 68, 72, 77, 78, 80, 81
Flowering: time (days after seed sowing)- 20 (MG)	Early <60 days (3)	18	22.22	2, 4, 5, 7, 10, 12, 13, 26, 31, 35, 36, 45, 46, 52, 53, 57, 61, 71
	Medium 60–80 days (5)	63	77.78	1, 3, 6, 8, 9, 11, 14, 15-25, 27-30, 32-34, 37-44, 47-51, 54-56, 58-60, 62-70, 72-81
	Late >80 days (7)	0	0.00	-
Fruit size: length/diameter ratio- 21-23 (MG & MS)	Small <1.0 (3)	1	1.23	51
	Medium 1.0–2.0 (5)	39	48.15	4, 7, 10, 12, 15, 17-27, 30, 32, 33, 38, 41, 43-47, 49, 50, 53, 55, 56, 62, 63, 66, 69, 70, 74, 76, 80
	Large >2.0 (7)	41	50.62	1-3, 5, 6, 8, 9, 11, 13, 14, 16, 28, 29, 31, 34-37, 39, 40, 42, 48, 52, 54, 57-61, 64, 65, 67, 68, 71-73, 75, 77-79, 81
Fruit: general shape-24 (VG)	Globular (1)	15	18.52	4, 12, 15, 17, 19, 25, 41, 45, 47, 49, 51, 56, 62, 66, 74
	Ovoid (2)	22	27.16	10, 21, 22, 24, 30, 32, 33, 38, 44, 50, 53, 55, 57, 63, 64, 68, 70, 72, 73, 78-80
	Obovate (3)	11	13.58	9, 11, 16, 18, 20, 26, 28, 34, 39, 43, 69
	Pear shaped (4)	2	2.47	23, 65
	Club shaped (5)	13	16.05	5, 14, 31, 35, 36, 40, 48, 52, 60, 61, 76, 77, 81
	Ellipsoid (6)	4	4.94	1, 3, 7, 71,
	Cylindrical (7)	14	17.28	2, 6, 8, 13, 27, 29, 37, 42, 46, 54, 58, 59, 67, 75

<i>Plant parts: Characters-serial numbers (type of assessment/stage of observation)</i>	<i>States (Observation note)</i>		<i>No. of cultivars</i>	<i>Per cent of cultivars</i>	<i>Cultivars code no.*</i>
Fruit: diameter of pistil scar- 25 (MS)	Small <1.0 cm (1)		75	92.59	1-7, 8-22, 24-36, 38-42, 44-47, 49-64, 66-75, 77-81
	Medium 1.0–1.5 cm (3)		0	0.00	-
	Large >1.5 cm (5)		6	7.41	23, 37, 43, 48, 65, 76
Fruit: Shape of apex- 26 (VS)	Indented (1)		31	38.27	1, 6, 7, 13, 14, 20, 22, 26, 27, 32, 33, 35-38, 40, 42, 50, 52, 54, 60, 63, 66, 67, 69-71, 77-79, 81
	Flattened (2)		11	13.58	3, 12, 15, 17, 23, 34, 45, 46, 51, 55, 74
	Rounded (3)		19	23.46	9, 10, 19, 25, 30, 41, 43, 44, 47-49, 53, 56, 62, 64, 65, 68, 73, 76
	Pointed (4)		20	24.69	2, 4, 5, 8, 11, 16, 18, 21, 24, 28, 29, 31, 39, 57-59, 61, 72, 75, 80
Fruit: curvature (only for cylindrical types)- 27 (VS)	Absent (1)		4	4.94	9, 23, 43, 48
	Slight (3)		11	13.58	5, 7, 29, 35, 37, 46, 57, 59, 61, 71, 77
	Medium (5)		10	12.35	2, 6, 8, 13, 27, 42, 52, 54, 75, 79
	Strong (7)		1	1.23	67
Fruit: color of skin at commercial harvesting- 28-30 (VG & VS)	White (1)		5	6.17	20, 38, 44, 62, 73
	Green (2)	Light (3)	1	1.23	63
		Medium (5)	3	3.70	8, 36, 71
		Dark (7)	14	17.28	4, 9, 18, 22, 29, 32, 41, 43, 48, 67, 70, 72, 74, 75
	Purple (3)	Light (3)	16	19.75	1, 3, 10-12, 14, 15, 17, 31, 33, 34, 45, 47, 51, 54, 81
		Medium (5)	20	24.69	16, 19, 24, 26, 28, 35-37, 40, 42, 46, 49, 58, 60, 64-66, 68, 69, 76, 78
Fruit: density of stripes-31 (VG)		Dark (7)	22	27.16	2, 5-7, 13, 21, 23, 25, 27, 30, 39, 50, 52, 53, 55-57, 59, 61, 67, 77, 80
	Absent (1)		67	82.72	1, 2, 4, 5, 7-9, 11, 13-16, 18-20, 22-26, 28-30, 32-42, 44, 46, 47, 49-56, 57-67, 69, 71-77, 80, 81
	Sparse (3)		6	7.41	12, 17, 31, 48, 54, 78
	Medium (5)		6	7.41	6, 10, 27, 43, 70, 79
	Strong (7)		2	2.47	21, 68
Fruit: patches- 32 (VG)	Absent (1)		43	53.09	2, 3, 5-8, 14, 19, 20, 23, 25, 26, 29, 30, 37-42, 44, 45, 47, 49, 50, 52, 54, 55, 57-59, 61-66, 69, 72, 73, 75, 76, 81
	Present (9)		38	46.91	1, 4, 9-13, 15-18, 21, 22, 24, 27, 28, 31-36, 43, 46, 48, 51, 53, 56, 60, 67, 68, 70, 71, 74, 77-80
Fruit: glossiness at harvest maturity-33 (VG)	Weak (3)		21	25.93	1, 6, 9, 12, 14, 17, 18, 22, 26, 29, 32, 34, 43, 46, 47, 49, 54, 64, 68, 70, 74
	Medium (5)		38	46.91	2, 3, 5, 8, 10, 11, 15, 19, 21, 31, 33, 35, 38, 40-42, 44, 45, 48, 51, 53, 55, 58, 60, 62, 63, 65, 66, 69, 71, 72, 73, 75-79, 81
	Strong (7)		22	27.16	4, 7, 13, 16, 20, 23, 24, 25, 27, 28, 30, 36, 37, 39, 50, 52, 56, 57, 59, 61, 67, 80
Fruit: size of calyx-34 (MS)	Small (3)		31	38.27	1, 7, 10, 13, 15, 16, 20-28, 31, 35, 36, 43, 49, 53, 56, 59, 60, 68, 73-75, 77, 81
	Medium (5)		43	53.09	2-6, 8, 11, 12, 14, 17, 19, 29, 30, 32-34, 37-39, 44-48, 50-52, 54, 55, 57, 58, 61-64, 66, 67, 69-72, 78-80
	Large (7)		7	8.64	9, 18, 40-42, 65, 76
Fruit: color of calyx and their intensity-35-36 (VG)	Green (1)	Weak (3)	17	20.99	7, 9, 13, 15, 22, 26, 27, 33, 35, 38, 40-42, 51, 60, 66, 75
		Medium (5)	44	54.32	1-3, 5, 6, 8, 10-12, 14, 15, 21, 25, 28, 31, 32, 34, 36, 37, 43-46, 48-50, 52-55, 57, 59, 61-65, 67, 68, 72, 76, 77, 78
		Strong (7)	10	12.35	4, 17, 18, 20, 29, 47, 70, 73, 74, 79
	Purple (2)	Weak (3)	4	4.94	16, 24, 71, 81
		Medium (5)	5	6.17	19, 30, 39, 69, 80
		Strong (7)	1	1.23	56

<i>Plant parts: Characters-serial numbers (type of assessment/stage of observation)</i>	<i>States (Observation note)</i>	<i>No. of cultivars</i>	<i>Per cent of cultivars</i>	<i>Cultivars code no.*</i>
Fruit: spininess of calyx-37 (VS)	Absent (1)	71	87.65	2-9, 10-25, 26, 27, 29-40, 42-46, 48-67, 69, 70, 75-81
	Weak (3)	5	6.17	1, 28, 47, 71, 73
	Medium (5)	2	2.47	41, 67
	Strong (7)	3	3.70	68, 72, 74
Fruit: ribs- 38 (VG)	Absent (1)	63	77.78	1-9, 13-16, 18-20, 22-27, 29-34, 36-40, 42, 43, 45, 48-50, 52-54, 57-65, 67-71, 75-81
	Weak (3)	10	12.35	10, 11, 21, 28, 47, 51, 55, 56, 73, 72
	Medium (5)	6	7.41	12, 17, 35, 41, 46, 66
	Strong (7)	2	2.47	44, 74
Fruit: creasing of calyx-39 (VG)	Weak (3)	30	37.04	1, 11, 13-16, 18, 20, 24, 28, 31, 35, 37, 38, 40, 42-48, 51, 52, 54, 64, 67, 69, 71, 81
	Medium (5)	31	38.27	2-4, 7, 9, 10, 12, 17, 19, 21, 23, 26, 32-34, 36, 39, 49, 53, 55, 57, 58, 60, 63, 65, 70, 72, 73, 75, 76, 78
	Strong (7)	20	24.69	5, 6, 8, 22, 25, 27, 29, 30, 41, 50, 56, 59, 61, 62, 66, 68, 74, 77, 79, 80
Fruit: color of flesh- 40 (VS)	Whitish (1)	18	22.22	4, 10, 18, 20, 23, 24, 28, 33, 37, 38, 41, 47, 49, 51, 68, 72, 74, 81
	Greenish (2)	63	77.78	1-3, 5-9, 11-17, 19, 21, 22, 25-27, 29-32, 34-36, 39, 40, 42, 44-46, 50, 52-64, 66, 67, 69-71, 73, 75, 77-80
Fruit: length of peduncle- 41 (MS)	Short <1.0 cm (3)	0	0.00	-
	Medium 1.0–5.0 cm (5)	45	55.56	1, 5, 7, 11, 13, 15, 17, 20-26, 28, 30-36, 44-47, 49-51, 53, 55, 60, 63, 64, 68, 69, 72-77, 79-81
	Long >5.0 cm (7)	36	44.44	2-4, 6, 8-10, 12, 14, 16, 18, 19, 27, 29, 37-43, 48, 52, 54, 56-59, 61, 62, 65-67, 70, 71, 78

* Name and codes of cultivars are given in supplementary Table S1 (Supp.).

Table S4 (Suppl.): Morphological characteristics of feature of crop ideotype in 81 extant cultivars of brinjal

<i>Plant parts: Characters-serial numbers (type of assessment/stage of observation)</i>	<i>States (Observation note)</i>	<i>No. of cultivars</i>	<i>Per cent of cultivars</i>	<i>Cultivars code no.*</i>
Fruiting: pattern-42 (VG)	Solitary (1)	49	60.49	4, 7-9, 11, 12, 14, 15, 17, 18, 19, 21, 22, 25-27, 28, 30, 32-36, 38-43, 45, 47, 49, 51-53, 55, 57, 60, 62-66, 69, 71, 72, 74, 76, 78
	Cluster (2)	14	17.28	1, 2, 6, 13, 20, 24, 29, 37, 46, 48, 50, 58, 75, 77
	Mixed (3)	18	22.22	3, 5, 10, 16, 23, 31, 44, 54, 56, 59, 61, 67, 68, 70, 73, 79-81
Plant: growth habit- 43 (VG)	Erect (1)	10	12.35	4, 8, 23, 31, 56, 62-65, 76
	Semi spreading (5)	28	34.57	1, 2, 6, 9, 11, 13, 20, 22, 32-34, 37, 39, 40, 43, 44, 46, 48, 49, 51, 52, 57, 60, 66, 67, 69, 71, 80
	Spreading (7)	38	46.91	3, 5, 7, 10, 12, 14-16, 18, 19, 21, 24-30, 35, 36, 38, 41, 42, 45, 47, 50, 53-55, 58, 61, 68, 70, 73, 75, 77-79
	Horizontal (9)	5	6.17	17, 59, 72, 74, 81
Plant: height- 44 (MG)	Very short <30 cm (1)	0	0.00	-
	Short 30–60 cm (3)	7	8.64	27, 28, 41, 72-75
	Medium 61-100 cm (5)	46	56.79	1, 2, 4-8, 10, 13, 16, 20, 21, 23, 24, 30, 31, 35, 37-39, 43-50, 52, 55-62, 67, 68, 71, 76-81
	Tall 101-150 cm (7)	28	34.57	3, 9, 11, 12, 14, 15, 17, 18, 19, 22, 25, 26, 29, 32-34, 36, 40, 42, 51, 53, 54, 63-66, 69, 70

<i>Plant parts: Characters-serial numbers (type of assessment/stage of observation)</i>	<i>States (Observation note)</i>	<i>No. of cultivars</i>	<i>Per cent of cultivars</i>	<i>Cultivars code no.*</i>
Plant: spread (distance between two extremes leaf tips at widest point- 45 (MG))	Narrow <50 cm (3)	5	6.17	9, 27, 41, 67, 72
	Medium 50-100 cm (5)	48	58.02	1, 2, 4-8, 10, 12, 16, 19, 20-25, 30, 35, 36, 38, 40, 44-47, 49, 50, 52, 53, 55-57, 59-63, 68, 69, 71, 73-75, 77-80
	Broad >100 cm (7)	28	35.80	3, 11, 13-15, 17, 18, 26, 28, 29, 31-34, 37, 39, 42, 43, 48, 51, 54, 58, 64-66, 70, 76, 81
Fruit: color of skin at physiological maturity- 46 (VS)	Yellow (1)	47	59.26	3, 4, 8, 9, 11, 13-15, 17, 18, 20-22, 24, 26, 28, 29, 31-40, 44, 45, 48, 49, 51, 53, 58, 60, 62-64, 66, 70-75, 79, 81
	Orange (2)	2	2.47	65, 76
	Brown (3)	32	38.27	1, 2, 5-7, 10, 12, 16, 19, 23, 25, 27, 30, 41-43, 46, 47, 50, 52, 54-57, 59, 61, 67-69, 77, 78, 80
Time of physiological ripeness (days after fruit set)- 47 (MG)	Early <65 days (1)	29	35.80	1, 3, 4, 5, 7, 8, 10, 13, 14, 21, 27-29, 31, 35, 36, 40-42, 52, 54, 58-61, 67, 71, 75, 81
	Medium 65-75 days (3)	15	18.52	6, 12, 15, 17, 19, 23, 25, 37, 43, 45-49, 51, 55, 56, 62, 66, 76
	Late >75 days (5)	37	45.68	2, 9, 11, 16, 18, 20, 22, 24, 26, 30, 32, 33, 34, 38, 39, 44, 50, 53, 57, 63-65, 68-70, 72-74, 77-80

*Name and codes of cultivars are given in supplementary Table S1 (Supp.)

RESEARCH ARTICLE

Assessment of Spine Gourd (*Momordica dioica* Roxb.) with Cross-species Transferable Microsatellite and ISSR Markers

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Abstract

Spine gourd (*Momordica dioica* Roxb.) is a highly nutritious and underutilized potential vegetable containing high amount of protein as compared to other cucurbitaceous vegetables. The present study was under maker to identify and develop the molecular markers for genetic diversity study in spine gourd. In this regard, total 94 (24 *M. dioica*, 50 *M. charatia*, 10 *Cucumis sativus*, 10 *C. melo*) nucleotide sequences were obtained from national center for biotechnology information (NCBI) platform followed by extraction of desirable sequences by Batch primer 3. Out of 94 simple sequence repeats (SSR) sequences, 52 were amplified with spine gourd genotype. However, only seven SSRs were polymorphic and useful for diversity study from the 52 markers. In addition, 10 ISSR markers were also evaluated for genotyping and genetic diversity study. SSR markers were produced 2.42 average alleles per primer, whereas inter simple sequence repeats (ISSR) markers were produced 4.6 average alleles per primer. The average polymorphism information content (PIC) value for SSR markers was 0.32 and for ISSR was 0.73. SSR markers based cluster analysis revealed two major cluster of 41 genotypes in which highest dissimilarity was obtained between genotypes PK-49 and NDM-3 at 60% Jaccard's similarity coefficient. Similarly, ISSR markers based diversity analysis also grouped the 41 genotypes into two clusters in which highest dissimilarity was recorded between PK-35 and PMD-5-1 at 66% dissimilarity level. Diverse genotypes will be very useful in cross-breeding programmes. Molecular markers identified through this study can be used for further crop improvement activities.

Keywords: Genetic diversity, Molecular markers, Polymorphism information content, Spine gourd, Transferability.

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Introduction

Spine gourd (*M. dioica* Roxb.) is an important potential vegetable crop and one of the most consumed during rainy season in India and East Asia. It belongs to the Cucurbitaceae family having chromosome no. $2n=2x=28$. This commercial vegetable crop is gaining popularity because it is rich in taste and has high nutritional value. Per 100 g of edible fruit was found to contain carbohydrate 47.92 g, crude protein 19.38 g, crude fiber 21.30 g, crude lipid 4.70 g and ash 6.70 g. It also contained little quantities of essential elements like iron 4.60 mg, phosphorus 42 mg, calcium 33 mg, vitamins like thiamin 0.05 mg, riboflavin 0.18 mg, niacin 0.06 mg, ascorbic acid 29.94 mg and carotene 162 mg (Bharathi *et al.*, 2007 and Aberoumand, 2011).

Spine gourd's medicinal and curative properties are known for ages and are passed on from generation after generation as traditional knowledge. Decoction of leaves reduces fever; tuberous roots help in relieving headaches, excess sweating, stone formation and migraine, while the fruit is quite helpful in controlling diabetes and blood pressure (Ram *et al.*, 2001, Talukdar and Hossain, 2014). Tuberous roots are used for curing diarrhea, fever and rheumatism by the tribal's of Chhattisgarh, seeds are used against chest problems and stimulate urinary discharge (Bharathiet *al.*, 2007).

Area under spine gourd cultivation has increased day by day because of so many advantages like high demand in the market good nutritional and medicinal value, high keeping quality, ability to withstand long-distance transportation, high market price and good export potential (Rasul, 2003). Thus, this minor vegetable crop has gained popularity among farmers and may be a useful supplement crop in developing nations, especially in overpopulation and undernourished areas. The initiation of any plant breeding programme requires the morphological characterization of germplasm but through morphological characterization, we limited for only few characters and genes.

In the recent era of Omics, all types of information viz., morphological, biochemical, physiological and molecular, were generated in huge quantities and in a very short period, leading to rapid selection and development of new genotypes with diverse characters. The development of molecular markers could complement the morphological markers. This enabled the plant breeders to overcome the limitations faced in the use of morphological markers (Rana and Das, 2016). The knowledge of morphological and molecular diversity in a crop species is essential for their improvement. The genetic diversity among the landraces might contribute to intra-specific crosses among this spine gourd collection landraces having high potential for genetic improvement of this vegetable crop. Spine gourd had great medicinal and nutritional value, but due to some problems like dioecious nature, low seed germination, availability of plant materials and early-stage identification of sex type restrict the domestication process. Recently, the transferability of SSR markers has also been reported in related species of spine gourd (Shukla *et al.* 2017) for understanding the genetic variations between species and genera. To accelerate the domestication of the spine gourd two major objectives were studied in this investigation, which are (1) Morphological characterization of diverse genotypes of spine gourd, and (2) Identification of molecular markers and their utilization for generation of molecular data of spine gourd germplasm.

Materials and Methods

Experimental Site

Morphological characterization of spine gourd genotypes were done at the research cum instructional farm and molecular markers-based characterization was accomplished at Plant Molecular Biology and Biotechnology laboratory of Rajmohini Devi College of Agriculture and Research Station, IGKV, Ambikapur (Chhattisgarh).

Plant Materials, Planting Geometry and Morphological Characters Recorded

The experimental plant material comprised of 41 genotypes of spine gourd, including two checks. These accessions

were originated from different agroecological regions of the country (Table 1). Plant materials were grown at a field in 13 blocks to evaluate morphological characters during the crop season of 2018-19.

The planting geometry between plants was 2 x2 m for each line. The ratio of female: male (F:M) was maintained at 8:1 ratio to obtain sufficient fruit yield. Standard agronomic practices were adopted during the entire crop period. Special mechanical supports were provided to plants through the bamboo sticks for better growth, development and fruit yield.

Total 18 characters, including plant morphology and fruit yield parameters, were recorded according to the standard method during different crop growth stages. Details of the morphological characters and their stage of observation are given in Supplementary Table 1.

Identification and Synthesis of SSR Marker based on Cucurbitaceous Crop Genome Sequence for Spine Gourd

Nucleotide sequences of cucurbitaceous crops viz., cucumber, bitter gourd, and watermelon were searched from NCBI platform to identify and synthesize SSR Markers for spine gourd. From such nucleotide sequences, FASTA sequences have been extracted for designing the SSR primers by using primer designing program *i.e.* Batch primer 3. As suggested by Castoe *et al.* (2012) for selecting SSR primer from FASTA sequence, standard criteria was applied during the designing of SSR primers by Batch primer 3. Following this procedure, total 94 (24 *M. dioica*, 50 *M. charatia*, 10 *C. sativus*, 10 *C. melo*) SSR primer sequences were obtained synthesized.

All 94 SSR primers were initially screened in a single genotype IK-1 of *M. dioica* to check their quality, annealing temperature, amplification and compatibility with the *M. dioica* genome. Based on this experiment, total 52 SSRs were amplified and showed genome compatibility with *M. dioica*. When these 52 markers were used for fingerprinting in 41 genotypes, only seven showed polymorphism. Therefore, only seven SSR markers were identified for fingerprinting and molecular diversity analysis in *M. dioica*. A list of seven SSR markers and name of their source genome and other details are given in Table 2.

ISSR Marker Identification

Total 10 ISSR markers were procured from R. H. Richharia Plant Molecular Biology Laboratory, Department of Genetics and Plant Breeding, IGKV, Raipur for the current study. Primers details are given in Table 3. All the ISSRs were screened in a genotype of *M. dioica* IK-1 to check their quality, annealing temperature, amplification and compatibility with *M. dioica* genome. All the 10 ISSR markers were amplified however, only seven ISSRs were polymorphic out of the 10 markers. Therefore, seven ISSR markers were identified and used for genetic diversity analysis.

Table 1: List of the genotypes of spine gourd used for the investigation along with their source of collection

S. No.	Genotypes	Sources
1	IK-1 (C)	Ambikapur, Chhattisgarh
2	RMDSG-3 (C)	Ambikapur, Chhattisgarh
3	PK-5	Surguja, Chhattisgarh
4	PK-9	Surguja, Chhattisgarh
5	PK-26	Surguja, Chhattisgarh
6	PK-34	Surguja, Chhattisgarh
7	PK-35	Surguja Chhattisgarh
8	PK-46	Surguja, Chhattisgarh
9	ASG-18-5	Ambikapur, Chhattisgarh
10	NDM-5	Faizabad, Uttar Pradesh
11	RMF-7-P-1	Rahuri, Maharashtra
12	RMF-P-4	Rahuri, Maharashtra
13	RMF-27	Rahuri, Maharashtra
14	RMF-17	Rahuri, Maharashtra
15	RMF-1	Rahuri, Maharashtra
16	PK-49	Surguja, Chhattisgarh
17	PK-33	Surguja, Chhattisgarh
18	PHULLE MD-5-2	Rahuri, Maharashtra
19	DHARAMJAYGARH	Dharmjaygarh, Raigarh, Chhattisgarh
20	PHULLE MD-5-1	Rahuri, Maharashtra
21	RMDSG-1	Surguja, Chhattisgarh
22	KRISHNAPUR	Surguja, Chhattisgarh
23	AMBIKA K-12-1	Pratappur, Balrampur
24	AMBIKA-13-5	Pratappur Mani, Balrampur, Chhattisgarh
25	AMBIKA-13-6	Pratappur, Balrampur
26	AJSG-3	Jashpur, Chhattisgarh
28	RMF-G-49	Rahuri, Maharashtra
29	NDM-1	Faizabad, Uttar Pradesh
30	NDM-4	Faizabad, Uttar Pradesh
31	NDM-3	Faizabad, Uttar Pradesh
32	NDM-2	Faizabad, Uttar Pradesh
33	RAIGARH	Raigarh, Chhattisgarh
34	AJSG-4	Jashpur, Chhattisgarh
35	AJSG-1	Jashpur, Chhattisgarh
36	AJSG-2	Jashpur, Chhattisgarh
37	AJSG-5	Jashpur, Chhattisgarh
38	ASG-18-1	Ambikapur, Chhattisgarh
39	ASG-18-2	Ambikapur, Chhattisgarh
40	ASG-18-3	Ambikapur, Chhattisgarh
41	ASG-18-4	Ambikapur, Chhattisgarh

DNA Extraction from Tender Leaves

DNA was isolated with the help of the modified CTAB method. Tender leaves free from diseases, insects, and viruses and developmental deformities, were collected from each genotype. Collected leaves were kept in ice box

and brought to the laboratory where they were washed with 70% ethanol to remove traces of dirt and used for DNA extraction. Theleaves were cut into small pieces and kept in mortar which have 400 µL CTAB and ground thoroughly with the help of pestles. Samples were transferred on 1-mL

Table 2: List of seven SSR markers, sequence, annealing temperature, PIC value and name of their source genome

Markers name	Forward sequence	Reverse sequence	No. of alleles produced	Alleles size (bp)	Alleles frequency	PIC value	Source genome
SgSSR1	CAGGGGCGATTAGATTATTC	GTGATGTGGGTCGAGCAGTA	2	100 180	0.95 0.05	0.095	<i>M. dioica</i>
SgSSR17	AGAGATAACCGCAGTTCATAA	TTCTCTCTTTCTCTCATCCA	3	100 200 800	0.64 0.2 0.15	0.521	<i>M. dioica</i>
SgSSR15	AGTTCTACTGAGATGGAAGTGG	TGTAAAAACCAGAACCTATGC	2	150 500	0.94 0.051	0.097	<i>M. dioica</i>
SgSSR13	ATTGGTCATCTCGAAAGGTAT	GTTGGAAAAATGTGGTAACAG	3	150 600 800	0.76 0.078 0.15	0.384	<i>M. dioica</i>
SgSSR11	CACATGATTTATGGGTTTCAT	TGAGTAAGAGAGAGAACGAAAA	3	50 100 400	0.57 0.24 0.18	0.58	<i>M. dioica</i>
McN12	CAGAGGGGTGGTTCCTCTTT	CCACATGGATGATCGAGAGA	2	100 250	0.71 0.28	0.404	<i>M. charantia</i>
McN24	CTCCAACCTGAGGAAAGAAAAC	AGAGCCAATTGGGGCTTTAT	2	50 100	0.91 0.088	0.162	<i>M. charantia</i>

Table 3: Details of ISSR markers used for genetic diversity study in spine gourd

S. No.	Primers	Sequence (5' – 3')	No. of alleles	Alleles size (bp)	Alleles frequency	Annealing temp. (°C)	PIC value
1	UBC-808	AGAGAGAGAGAGAGAGC	4	150	0.26	50	0.748
				200	0.26		
				250	0.25		
				350	0.21		
2	UBC-812	GAGGAGGAGGAGGAGAA	4	100	0.28	52	0.734
				200	0.28		
				280	0.28		
				450	0.14		
3	UBC-824	TCTCTCTCTCTCTCG	5	150	0.32	52	0.725
				220	0.32		
				300	0.025		
				400	0.24		
4	UBC-840	GAGAGAGAGAGAGAGAYT	4	500	0.10	52	0.712
				120	0.36		
				210	0.16		
				310	0.14		
5	UBC-841	GAGAGAGAGAGAGAGAYC	6	450	0.32	52	0.786
				120	0.26		
				200	0.25		
				250	0.20		
6	UBC-856	ACACACACACACACAYA	4	300	0.17	51	0.675
				350	0.08		
				400	0.01		
				180	0.35		
7	UBC-873	GACAGACAGACAGACA	5	280	0.34	51	0.734
				250	0.17		
				300	0.28		
				150	0.32		
				250	0.30	51	
				400	0.22		
				450	0.11		
				600	0.02		

Eppendorf tube and again 400 µL CTAB was added then kept in the water bath for 20 minutes at 65°C. After incubation, 700 µL of 24:1 chloroform: Iso-amyl alcohol was added and mixed well by using vortex. Later tubes were centrifuged at 14,000 rpm for 5 minutes to settle down the debris. Supernatant was taken out into new 1.5 mL centrifuge tube with the help of micro pipettes. Steps 5 and 6 were repeated one more time. Then 900 µl of chilled ethanol was added and kept for incubation at -20°C for 2 hours. After incubation, tubes were centrifuged at 14,000 rpm for 20 minutes and the supernatant was discarded. DNA pellet was washed with 50 µL of 70% ethanol. Tubes were centrifuged for 3 minute at 14,000 rpm and upper layer of ethanol was discarded. Pellet was dried through air at room temperature. 100 µL of TE buffer was added to dissolve the pellet.

Table 4: PCR mix of 20 µL volume was prepared in 1.5 mL centrifuge tube by using the following components

Stock	Final Concentration	Volume (µL)
Sterile ddH ₂ O	-	11.5
PCR buffer	10 X	2.5
dNTPs (mix)	1.0 mM	1.5
Primer (forward)	5 pmol.	1
Primer (reverse)	5 pmol.	1
DNA Template	100 ng/µL	2
Taq polymerase	5 U/µL	0.5
Total	-	20

Quantification of DNA

Quality and quantity of DNA were checked by electrophoresis on 1% agarose gel. Total 6 µL of solution was prepared by adding 3 µL of extracted DNA and 3 µL of 6X loading dye (bromo phenol blue). The solution was mixed well and vortexed to settle down into the bottom and loaded into the wells of agarose gel. Current of 50V was applied till the initial movement of DNA from the wells and later increased to 70V. Once the tracking dye reached to 90% length of gel, electrophoresis was stopped and the visualized in gel documentation system E-BOX CX5.TS (20M). A single intact band of genotype indicated the good quality and quantity of DNA. Based on the band intensity, extracted DNA was diluted and further used for PCR amplification by using SSR and ISSR markers.

PCR Amplification

PCR mix of 20 µL volume was prepared in 1.5 mL centrifuge tube by using several components as shown in Table 4. PCR cycle consisted of initial denaturation at 94°C for 5 minutes; followed by 40 cycles of amplification, at 94°C for 30 seconds (denaturation), 50 to 60°C for 1-minute (annealing) and 72°C for 1-minute (extension). A final extension step at 72°C for 7 minutes was followed by termination of the cycle and stored the PCR product at 4°C.

Agarose Gel Electrophoresis and Visualization

A total of 1% agarose gel (1-gm agarose + 100 mL 1X TBE + 2 µL ethidium bromide) was used for separation and

Table 5: Descriptive statistics of various morphological characters in spine gourd genotypes

S. No.	Characters	Mean	Range		Standard Deviation	Coefficient of Variation (%)
			Minimum	Maximum		
1	Days to 1 st flowers	41.20	32.00	54.00	5.82	14.14
2	No. of first flowering node	14.85	6.00	24.00	5.08	34.21
3	No. of stem per plant	10.32	5.00	18.40	3.57	34.61
4	Leaf Length (cm)	7.35	5.34	9.60	0.97	13.17
5	Stem color	1.15	1.00	2.00	0.36	31.22
6	Leaf width (cm)	7.20	5.58	9.10	0.83	11.51
7	Pedicle length (cm)	2.91	1.52	4.08	0.52	17.79
8	Ovary length (mm)	1.70	1.40	2.44	0.24	14.27
9	Ovary diameter (mm)	0.71	0.52	0.86	0.07	10.24
10	Style length (mm)	0.77	0.62	1.02	0.09	12.04
11	Pistil length(mm)	0.60	0.40	0.76	0.10	16.48
12	Fruit length (cm)	4.19	2.84	5.96	0.69	16.53
13	Fruit diameter (cm)	3.31	2.36	4.56	0.64	19.40
14	No. of fruit per plant	112.28	103.20	121.20	4.49	4.00
15	Single fruit weight (g)	14.78	9.60	19.00	2.52	17.05
16	Fruit yield per plant (kg)	1.67	1.07	2.52	0.30	18.14
17	No. of seed per fruit	9.77	7.80	11.20	0.80	8.19
18	100 Seed weight (g)	26.52	14.50	41.00	7.03	26.49
19	Fruit yield (q/ha)	33.37	21.35	50.42	6.05	18.14

Table 6: List of top performing three genotypes for fruit yield and related traits

S. No.	Name of traits	Genotype-1	Genotype-2	Genotype-3
1	Days to 1 st flowers	IK-1 (32)	PK-34 (33)	ASG-18-4 (34)
2	Leaf length (cm)	PK-9 (9.6)	PK-34 (8.8)	RMF-7-P-1 (8.74)
3	Fruit length (cm)	PK-49 (5.96)	PK-26 (5.88)	RMF-7-P-1 (5.68)
4	Fruit diameter (cm)	PK-33 (4.56)	AMBIKA 13-6 (4.32)	RMF-G-39 (4.22)
5	No. of fruit per plant	ASG-18-5 (121.2)	RMF-1 (121)	IK-1 (120.4)
6	Single fruit weight (g)	AMBIKA 13-5 (19)	KRISHNAPUR (18.6)	IK-1 (18.4)
7	Fruit Yield per plant (kg)	RMDSJ-1 (2.52)	IK-1 (2.22)	AMBIKA 13-5 (2.09)
8	No. of seed per fruit	NDM-1 (11.2).	AMBIKA 13-5 (11.2)	RMF-G-39 (11.2)
8	100 Seed weight (g)	ASG-18-1 (41)	PK-35 (38.54)	ASG-18-2 (38.5)
9	Fruit yield (q/ha)	RMDSG-1 (50.42)	IK-1 (44.31)	AMBIKA 13-5 (41.88)

visualization of PCR products. In 2 µL loading dye (10x) was added to PCR products and mixed well. In 10 µL of PCR mix were loaded into the wells. Total 50 bp DNA ladder was loaded in the first well. Gel was run at 70 volts for SSR and 80 volts for ISSR for 40 minutes till the dye reached to the last end of the gel. Thereafter, gel was visualized by E-BOX CX5.TS (20M) having UV Trans-illuminator and gel pictures were saved for further scoring of bands.

Scoring of Bands

The banding pattern of genotypes, developed by each set of primer was scored separately. If band is present for a specific amplicon size it was scored as '1' (allele is present) and absence of any band for that position was scored as '0' (allele is absent). Allele size of different markers was determined based on the banding pattern of 50 bp DNA ladder.

Bio-statistical Analysis of Morphological and Molecular Data

Morphological data obtained from 5 plants of each genotype were used for descriptive statistics analysis by using the XLSTAT v18.3 software. Mean data were used for genetic diversity study by following and UPGMA (Un-weighted Pair Group Method with Arithmetic averages) algorithm and Euclidean distance by using XLSTAT v18.3 software.

Scoring of banding pattern of SSR and ISSR markers were used for genetic diversity analysis by following the UPGMA algorithm and Jaccard's similarity coefficient by using NTSYS-pc software. A dendrogram showing the distance-based relationship between the genotypes was constructed by following this method.

Results and Discussion

Performance of Genotypes based on Morphological Traits

Descriptive statistical parameters of various morphological traits are given in Table 5 and list of top performing three genotypes for fruit yield and related traits are given in Table

6. Days to 1st flowering ranged from 32 days IK-1 to 54 days RMF-27 from date of sowing with an average 41 days and standard deviation 5.68 days. Coefficient of variation for days to 1st flowering was 14.14%. Genotype IK-1 showed earliest flowering followed by genotype PK-34. Leaf length ranged from 5.34 to 9.60 cm with average 7.35 cm and standard deviation 0.97 cm. Coefficient of variation for leaf length was 13.17%. Genotype PK-9 had longest leaf followed by genotype PK-34. Fruit length was ranged from 2.84 to 5.96 cm with an average of 4.19 cm and standard deviation 0.69cm. Coefficient of variation for the trait is 16.53%. Genotype PK-49 had longest fruit followed by genotype PK-26. Fruit diameter was ranged from 2.36 to 4.56 cm with an average of 3.31 cm and standard deviation 0.64 cm. Coefficient of variation for the trait is 19.40%. Genotype PK-33 had highest fruit diameter followed by genotype Ambika 13-6. Number of fruits per plant ranged from 103 to 121 with an average of 112 and standard deviation 4.49. Coefficient of variation for the trait is 4%. Genotype ASG-18-5 had the highest number of fruits followed by genotype RMF-1 and genotype IK-1. Weight of single fruit ranged from 9.60 g to 19 g with an average of 14.78 g and standard deviation 2.52 g. Coefficient of variation for the trait is 17.05%. Genotype Ambika13-5 had highest single fruit weight followed by genotype Krishnapur. Fruit yield per plant ranged from 1.07 to 2.52 kg with an average of 1.67 kg and standard deviation 0.30 kg. Coefficient of variation for the trait is 18.14%. Genotype RMDSG-1 had highest fruit yield per plant followed by genotype IK-1. Number of seeds per fruit ranged from 7.80 to 11.20 with an average of 9.77 and standard deviation 0.80. Coefficient of variation for the trait is 8.19%. Genotype NDM-1 had the highest number of seeds per fruit followed by genotype AMBIKA 13-5. Hundred seed weight ranged from 14.50 to 41 g with an average of 26.52 g and standard deviation 7.03 g. Coefficient of variation for the trait is 26.49 %. Genotype ASG-18-1 had highest single fruit weight followed by genotype PK-5. Ample amount of variability is present among the genotypes that can be utilized for improvement of this crop.

Genetic Diversity based on Morphological Traits

Morphological character based genetic diversity analysis was performed to construct a dendrogram by following Euclidean distance and UPGMA algorithm through Sim Qual program NTSYSpc software version 2.02 (Rohlf, 1998) (Figure 1). All the 41 genotypes were grouped into two major clusters in which cluster-I have 40 genotypes whereas cluster-II have 1 genotype (AJSG-1). The Euclidean distance ranged from 0 to 15. In pairwise comparison, the maximum distance was obtained between AGSG-1 and PK-46 with the Euclidean distance of 15, whereas Raigarh and RMF-G-39 showed least distance (ED-5). Cluster-I further partitioned into two sub clusters in which first sub cluster consisted 30 genotypes whereas Sub cluster-II has consisted 10 genotypes.

Although, the dendrogram generated from similarity or genetic distance matrix based on morphological traits has provided an overall pattern of variation as well as the degree of relatedness among genotypes. Genotypes showing maximum dissimilarity could be used in hybrid breeding programs for developing good hybrids or for developing better transgressive segregates. Results obtained through current study have been corroborated by the outcomes of by Bhagat *et al.* (2017) on spine gourd. Similar morphological based cluster results were obtained by Singh *et al.* (2016) in cucumber.

Informative ness of SSR Markers and their Utilization in Genetic Diversity Study

A total of 52 SSR primers (9 Cucumber, 6 Muskmelons, 19 Spine gourd and 18 Bitter gourd) were amplified from which only seven SSR primers were found to be polymorphic. A total of 17 alleles were produced by 7 SSR primers with an average of 2.42 alleles per primer. Number of alleles amplified for each primer ranged from 2 to 3. The polymorphic information content (PIC) for these primers were ranged from 0.097 (SgSSR15) to 0.580 (SgSSR11) with average PIC 0.32 (Table 2).

The microsatellite primer SgSSR11, SgSSR13, SgSSR17 generated three alleles while SgSSR1, SgSSR15, McN12 and McN24 exhibited two polymorphic alleles. The PIC provides an estimate of discriminating power of a marker based on the number of alleles at a locus and relative frequencies of these alleles. Seven primers produced 361 bands across the 41 varieties and of which 123 were polymorphic (34%). Similar type result was obtained by Jacob *et al.* (2016) in bottle gourd landraces; Cui *et al.* (2017) in bitter gourd.

SSR markers based cluster analysis was performed by following the Jaccard's similarity coefficient and UPGMA algorithm to generate a dendrogram for 41 varieties which is being presented in Figure 2. Total genotypes were grouped into two major clusters viz., cluster-I and cluster-II. Highest dissimilarity was obtained between PK-49 and NDM-3. The similarity coefficient ranged from 0.60 to 0.95.

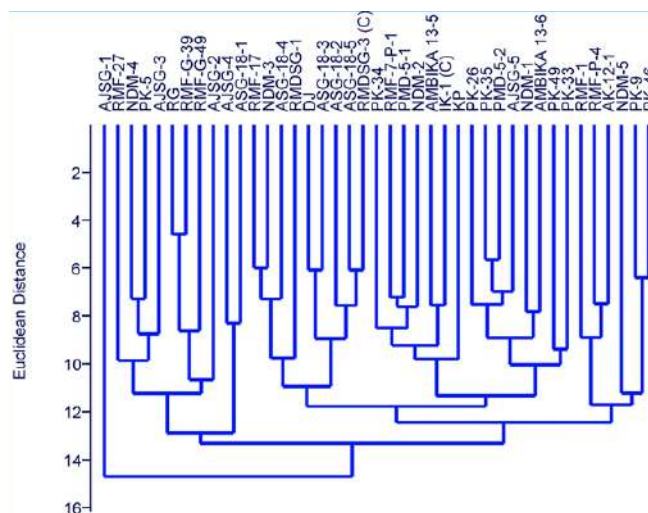


Figure 1: Morphological character based dendrogram depicting the relatedness among the 41 genotypes of spine gourd

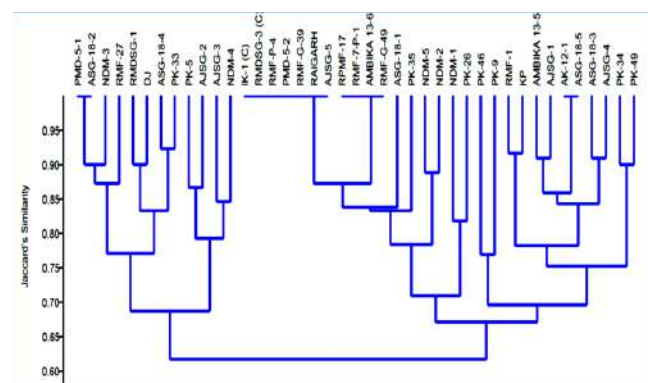


Figure 2: SSR marker based dendrogram depicting the relatedness among the 41 genotypes of spine gourd

According to pairwise comparison, the maximum similarity (100%) was obtained among the genotypes namely, IK-1, RMDSG-3, RMF-P-4, PHULLE MD-5-1, RMF-G-39, RAIGARH & AJSG-5; RPMF-17 & RMF-7-P-1, AMBIKA 13-6, RMF-G-49; AMBIKA-K-12-1 & ASG-18-5 in cluster-I whereas PHULLE MD-5-1 and ASG-18-2 in cluster-II. Cluster-I consisted of 29 varieties whereas cluster-II consisted of 12 genotypes. Highest dissimilarity in clustered-I was recorded between ASG 18-1 and PK-49 whereas highest dissimilarity in cluster-II was recorded between NMD-4 and NMD-3. Similar kind of results were also found by Yang *et al.* (2015) while analyzing the 42 Chinese accessions (mean similarity coefficient= 0.76) of cucumber. Dissimilar genotypes obtained through SSR marker based diversity analysis will be useful in recombination breeding to obtain the maximum heterosis for fruit yield per plant.

Informative ness of ISSR Markers and their Utilization in Genetic Diversity Study

Seven ISSR markers were used for discriminating 41 genotypes and all ISSR primer were found to be polymorphic.

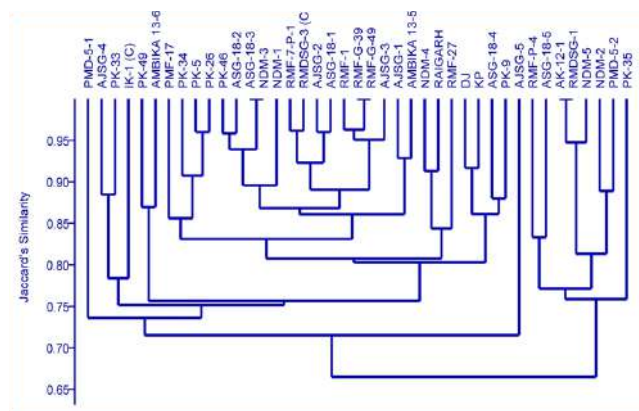


Figure 3: ISSR marker based dendrogram depicting the relatedness among the 41 genotypes of spine gourd.

Total 32 alleles were produced by the seven ISSR primers with an average 4.6 alleles per primer. The number of alleles amplified for each primer pair ranged from 4 (UCB-808, UCB-812, UCB-840, UCB-856) to 6 (UCB-841) and the polymorphic information content (PIC) for these primers ranged from 0.675 to 0.786 with an average PIC value of 0.73 (Table 3). PIC is a parameter that refers to the value of a marker for detecting the degree of polymorphism within a population. To determine PIC values of each ISSR primer we analyzed the mean of PIC values for all loci of each ISSR primer. It is assumed that a $PIC > 0.5$ accounts for a highly informative marker, $0.5 > PIC > 0.25$ for an informative marker, and $PIC < 0.25$ for a slightly informative marker (Botstein *et al.* 1980). A total of 932 bands amplified, of which 645 were polymorphic bands (69.20%). Similar results obtained by Singh *et al.* (2016) through genetic diversity by using ISSR markers in 11 cucumber genotypes.

ISSR marker based cluster analysis was performed by following the Jaccard's similarity coefficient and UPGMA algorithm to generate a dendrogram for 41 varieties which is being presented in Figure 3. All the 41 genotypes were grouped into two major clusters viz., Cluster-I and Cluster-II. The similarity coefficient ranges from 0.66 to 100. Highest dissimilarity was recorded between PK-35 and PMD-5-1 at 66% dissimilarity level. Cluster-I consisted 33 genotypes whereas cluster-II have 8 genotypes. In cluster-I, highest similarity was obtained between ASG-18-3 & NDM-3; RMF-G-49 & RMF-G-49 (100%) and highest dissimilarity recorded between AJSG-5 and PHULLE MD-5-1. Cluster-II consisted 8 varieties where the highest similarity was recorded between AMBIKA-K-12-1 and RMDSG-1 (100%) whereas highest dissimilarity obtained between PK-35 and RMF-P-4. Cluster-I further classified two sub-clusters, whereas sub-cluster-I consisted 32 genotypes whereas sub-cluster-II has one genotype (AJSG-5). Highest dissimilarity recorded between PHULLE MD-5-1 and PK-9 genotypes in sub-cluster-I. Similar results were found by Ehsan *et al.* (2015) in *C. melo* L. and by Singh *et al.* (2016) in cucumber genotypes.

Conclusion

This study reported morphological characterization and novel molecular markers to develop a unique identity of spine gourd germplasm. The cross-species transferable microsatellite were detected from bitter gourd, cucumber and musk melon in spine gourd and it will provide a platform for comparative mapping to understand genomic relationships among these four species. A total of 94 microsatellites and 10 ISSR markers were used, but only 7 microsatellites and 7 ISSR were found polymorphic and useful for diversity study. Though, it is suggested that genotyping by sequencing of spine gourd germplasm will aid in development of new markers and precise molecular characterization. Information generated from GBS could be applied for comparative mapping, map construction, marker-assisted trait selection and provide important marker data, which is limited in this potential vegetable crop. The genetic diversity among the landraces might contribute to intraspecific crosses among landraces of this spine gourd collection having high potential for genetic improvement of this vegetable crop.

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Supplementary Table 1: Details of the morphological characters and their stages of observation.

S.N.	Characteristics	Parameters	Codes	Stages of observation
1.	Days to first flowering	Early (<40 days)	1	I
		Medium (41–50 days)	3	
		Late (>50 days)	5	
2.	Number of first flowering node	5–10 nodes	1	I
		11–15 nodes	2	
		16–20 nodes	3	
		21–25 nodes	4	
3.	Stem colour	Light green (L.G)	1	I
		Green (G)	3	
		Dark green (D.G)	5	
4.	No. of stem per plant	Few (<10 stems)	1	I
		Moderate (11–15 stems)	5	
		Many (>15 stems)	9	
5.	Leaf length (cm)	Short (<6.5 cm)	1	I
		Medium (6.6–8 cm)	5	
		Long (>8 cm)	9	
6.	Leaf width (cm)	Narrow (1–6.5 cm)	1	I
		Medium (6.6–8 cm)	5	
		Wide (>8 cm)	9	
7.	Pedicel length (cm)	Short (1–2.5 cm)	1	I
		Medium (2.6–3.5 cm)	5	
		Long (>3.6 cm)	9	
8.	Ovary length (cm)	Short (>1–10 mm)	1	I
		Medium (11–20 mm)	5	
		Long (>20 mm)	9	
9.	Ovary diameter (cm)	Small (<7 mm)	1	I
		Large(>7.1 mm)	5	
10.	Style length (cm)	Short (<7 mm)	1	I
		Medium (7.1–9 mm)	5	
		Long (>9.1 mm)	9	

S.N.	Characteristics	Parameters	Codes	Stages of observation
11.	Pistil length (cm)	Short (<4 mm)	1	I
		Medium (4.1–6.0 mm)	5	
		Long (>6 mm)	9	
12.	Fruit length (cm)	Short (<3.0 cm)	1	II
		Medium (3.0–4.0 cm)	3	
		Long (>4.1 cm)	5	
		Very long(>51)	7	
13.	Fruit diameter (cm)	Small (<30 mm)	1	II
		Medium (31–40 mm)	5	
		Large (>40 mm)	9	
14.	No. of fruits per plant	Few (<100)	1	II
		Moderate (100–120)	5	
		Many (>120)	9	
15.	Single fruit weight (g)	Light (1–10 g)	1	II
		Medium (11–15 g)	5	
		Heavy (>15 g)	9	
16.	Fruit yield per plant (kg)	Low(<2 kg)	1	II
		Medium (2–3 kg)	3	
		Good (>3 kg)	5	
17.	No. of seed per fruit	Less (<5)	1	III
		Medium (5–10)	5	
		Many (>10)	9	
18.	100 Seed weight (cm)	Light (<20 g)	1	III
		Medium bold (20–40 g)	3	
		Bold (>40 g)	5	
19.	Fruit yield (q/ha)	Low (<10 q/ha)	1	III
		Medium (11–20 q/ha)	3	
		Good (21–30 q/ha)	5	
		Very good (31–40 q/ha)	7	
		Bumper (>41 q/ha)	9	

RESEARCH ARTICLE

Identification of Late Maturing and Rain Tolerant Promising Genotype in Date Palm (*Phoenix dactylifera* L.) in Hot Arid Region

Rama S. Singh*, Ram K. Meena, Brijash D. Sharma, Rakesh Bhargava¹ and Pyre L. Saroj

Abstract

Date palm (*Phoenix dactylifera* L.) is most suitable drought hardy fruit tree of hot arid region of the country. Date palm fruits are harvested in our country at doka (khalal) stage due to early rains. Normally date palm fruits get spoiled by rains at maturity stage. With this objective, work on the identification of rain tolerant and late maturing genotype in date palm was carried out at ICAR-CIAH, Bikaner. On the basis of 5 years of evaluation study (2015–2019), one elite genotype (CIAH/DP/Sel.-01) of red color berry with average 45 to 50 kg fruit yield/tree was marked promising and fruits harvested at doka stage at the end of August and first week of September. Since the fruits were harvested after rainy season, they did not show any damage by rain. The length of bunch is about bigger in size (80–95 cm.), bunch weight 4 to 6 kg.; number of bunch 9–12; no. of berries/strands 24–28; fruit size 3.20 x 2.20 cm.; fruit weight 9 to 12 g and TSS 34–38.0 brix. The IC number 0624544 was obtained from NBPGR, New Delhi for specific traits. The fruit is red in color, sweet in taste and edible at the doka stage. Besides fresh consumption, it can be used for making value added products. The promising genotype would be source of gene for further breeding programme.

Keywords: Date palm (*Phoenix dactylifera*), Late maturity, Red color, Rain tolerant, Genotype, Arid region.

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Introduction

Date palm (*Phoenix dactylifera* L.; Family- Arecaceae) also known as *Khajoor*, *kharek* is an ancient cultivated fruit tree of semi-arid and arid regions of the world. It grows well under poor desertic soils due to its hardy plant characteristics and deep root system. There is a well-known phrase for date palm: its feet in water and head in fire/sun. It requires dry hot climate for growth and development of fruits. In date palm growing countries, there is no rain during fruit maturity and the ripening period from July to September. However, Medjool cultivar has reported intermediate tolerance to rains since it is a late maturing variety (Zaid, 1995). Date palm can be grown successfully at such places where adequate irrigation facility is available besides dry hot climatic conditions.

Date palm crop has industrial significance in the country and its cultivation should be increased in potential areas. Fruits are used to prepare soft date, dry date, RTS, squash, pickle, chutney, wine, bakery products, etc. All parts of date palm are useful since its history of cultivation in several ways to make handicraft items (Chao and Krueger, 2007). Its seed kernel is also used for cattle, poultry, fish feed, etc.

All the commercial date palm cultivars have been developed through the selection of chance seedlings based on local needs. In Rajasthan, plantation of tissue culture plants in about 850 ha in

districts of western Rajasthan. Due to less production, there is good demand for date fruits and products in the country. In India, date palm production is not meeting our domestic demand and dates are imported from date palm- growing countries. Keeping the above in view, the study was carried out to identify the rain tolerant and late maturing genotype for hot arid region.

A field survey of date palm orchards was conducted in 2015 in Bikaner to identify genotypes with late maturity, rain tolerance and better fruit characters. The survey was done during flowering/fruiting period at date palm block. Late maturity was observed in a plant consecutive during three-four years. Color turning stage occurred at the end of July and doka stage of maturity was noted in the last week of August and first week of September. When maximum date palm varieties were harvested. A tree with prolific bearing, attractive fruit color, sweet in taste and disease free was marked. The vigorous growth of plant with erect stem, 12 to 13 feet high and length of leaf size 2 to 2.6 m was recorded, however, the number of sucker was low.

Data on an important bunch and fruit characters like spathes emergence, opening, number of bunches, length of bunch, no. of fruits/strand, fruit weight, fruit size, stone weight, size, TSS, acidity, tannin contents were recorded. The conservation of unique germplasm is being done at the Institute farm for further study.

The data pertaining to morphological and physico-chemical characters of fruit of date palm are presented in Table 1. The date palm elite type seedling plant growing on the boundary of the field was marked for observation and data on different morphological parameters were recorded. The height of plant was 12–13 feet and a number of suckers was less. The plant's growth was vigorous and prolific bearing habit, which might be due to fewer suckers and good numbers of leaves and crown formation. The fruit color was red and length of bunch was likely similar to cv. Sewi. However, the shape and size of fruit differed from red fruited commercial cv. Khuneizi, Sewi and Dayari (Figure 1). It seems the variation among the genotypes. The data on emergence

of spathes and opening exhibited that this is a medium to late maturing type. Manual pollination was done every year just after opening of spathes. Fruit set was about 90 to 95% which shows that this genotype is very compatible to pollination with pollen grains of local male type. The length of strand was also bigger like Sewi and number of berries per strand was 24 to 28. The number of berries was at par with yellow color variety Zahidi. The size of berry was recorded 3.20 to 3.40 cm length and 2.2 to 2.30 cm width and weight 9 to 12 g. It could be a better genotype from higher pulp content point of view for processing purpose. The size of stone was small having weight of 0.52 to 0.81 g. This fruit character is highly suitable for value added products. The weight of bunch ranged from 4 to 12 kg varied from year to year. The number of bunch was recorded 9 to 12 every year. The fruit yield potential was observed from 45 to 60 kg/tree. Fruits were harvested from end of August to first week of September. Varieties of date palms differ in their susceptibility to rain and humidity. This genotype differed with other red color varieties in respect of shape, size and maturity of fruits. It is mentioned that very early and late maturing varieties escape rains and consequently, their fruits are unaffected. The weather data of Bikaner presented in Table 2 showed that rains occur during the months of June, July, August and very little rain in September. The genotype matures after rainy period in the first week of September by this time harvesting is over under Indian conditions. Similarly, the following cultivars are reported to be most tolerant of humidity and rain: 'Halawy', 'Khadrawy', and 'Kaktoom'. The cultivar 'Medjool' is intermediate in its tolerance of humidity and rain (Zaid, 1995). However, there are no rains during fruit growth, maturity and ripening in date palm-growing countries in the world from May to October.

One bunch was left over on the tree to see the further maturity *pind* stage. The berry was found good up to end of September and there after spoilage of fruits started on the tree. The insect infestation was not observed in this genotype.



Figure 1: Bearing tree and berries of rain tolerant and late maturing genotype of date palm.

Table 1: Characters of late maturing, rain tolerant date palm (CIAH/DP/S-01) genotype

S. No.	Characters	2015	2016	2017	2018	2019
1	Date of spathe emergence	23.2.15	18.2.16	20.2.17	22.2.18	28.2.19
2	Spathe opening	14.3.15	11.3.16	16.3.17	18.3.18	25.3.19
3	Pollination	15.3.15	12.3.16	17.3.17	19.3.18	26.3.19
4	No of bunches/tree	10	9	10	12	17
5	Length of bunch (cm)	70.00	75.00	85.00	95.50	106.00
6	No of berries/strands	27	24	28	28	25
7	Length of strand (cm.)	60.5	62.0	65.4	67.3	65.5
8	Fruit weight (g)	12.24	10.90	10.50	10.53	10.10
9	Size of fruit (length & width cm)	3.30 x 2.33	3.20 x 2.20	3.30 x 2.22	3.40 x 2.30	3.31 x 2.030
10	Stone wt (g)	0.52	0.54	0.53	0.58	0.81
11	Stone size (length x width ,cm)	1.87 x 0.70	1.82 x 0.69	1.97 x 0.72	1.86 x 0.66	2.10 x 0.83
12	Date of harvesting	2.9.15	4.9.16	8.9.17	29.8.18	4.9.19
13	Av. wt. of bunch (kg.)	4.50	5.00	5.20	6.30	3.10
14	Yield (kg)	42.50	44.60	50.50	62.00	46.50
15	TSS 0 Brix	34.0	30.2	38.0	38.3	30.2
16	Acidity (%)	--	0.22	0.14	0.13	0.21
17	Ascorbic acid (mg per 100 g sample)	--	--	4.00	5.06	4.08
18	Tannins (mg/100 g)	--	---	2.40	3.26	2.58

Table 2: Rainfall (mm.) pattern during fruiting season in hot arid region (Bikaner, India)

S. No	Year/month	Jan.	Feb.	March	April	May	June	July	August	Sept.
1.	2015	0.00	22.0	35.0	52.20	73.10	54.60	118.70	128.10	0.00
2.	2016	0.00	4.90	21.0	0.00	0.00	79.00	90.60	134.80	0.00
3.	2017	2.20	0.00	0.80	0.00	19.20	0.00	29.30	90.60	6.00
4	2018	0.00	0.00	1.40	4.20	5.60	54.30	189.80	54.80	0.00
5.	2019	2.70	0.00	1.80	31.00	9.00	12.80	40.60	128.20	16.20

At color turning stage, pulp was hard and astringent in taste possibly due to tannin content. TSS of the fruit recorded at doka stage varied from 34 to 38 °Brix. The total sugar estimated at doka stage during 2018 on fresh weight basis was 490.96 mg/100 g; reducing sugars 232.06 mg and non-reducing sugars (258.9 mg/100g). However, acidity was recorded 0.028% at doka stage which varied according to maturity stages.

The doka stage fruit of late maturing genotype of red color berry was also tried to prepare soft date by dipping in boiling water for 45 and 90 seconds and then drying during the year 2018. Soft date was prepared with a recovery percentage of 50 to 56% and the organoleptic test showed that 90 second dipping treatment was suitable for softness and sweetness in soft date (*pind*). However, the appearance was dark reddish color in soft date since its berry color is red.

The study concluded that this selected palm is a unique germplasm of specific traits like late maturing, rain tolerant, having red color berry. This promising date

palm genotype will be the gene source for rain tolerance for future crop improvement programme. Moreover, rain-tolerant varieties are not available for cultivation under such climatic conditions. Further, it will also be useful for doka fresh fruit availability during the month of September in hot arid conditions. Besides fresh consumption, fruits will be processed in to soft and dry date.

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RESEARCH ARTICLE

Genetic Diversity Analysis for Seed Yield Attributing Traits in White Jute (*Corchorus capsularis* L.)

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Abstract

The current study analyzed the genetic diversity of 47 white jute genotypes based on eight seed yields and its contributing characteristics. The result indicated that 47 genotypes (indigenous, exotic and improved type) were grouped into five clusters based on Mahalanobis' D² analysis. Cluster I had the most genotypes (43), whereas clusters II, III, IV and V each had only one genotype. Seed yield contributed the most to diversity (47.27%), followed by pod length (12.21%). The germplasm line CIJ-44 produced the highest seed yield of 31.05 g per plant with 64.5 pods per plant, pod length of 9.75 cm and number of seeds per pod of 31.85. The first four principal components accounted for 78.09% of the total variance, indicating a high correlation among the characters investigated as per the dendrograms of cluster analysis and PCA. The first principal component contributed to 28.43% of the variation and was of the utmost significance. Accordingly, in PC1, plant height and pod length traits were important in dividing the genotypes for the sake of their high loadings.

Keywords: Diversity analysis, Seed yield, White jute.

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Introduction

Jute is a natural vegetable fibre crop with a diploid chromosome number $2n=2x=14$. It belongs to the malvaceae family (Benor, 2018) of the *Corchorus*, where secondary phloem fibres are obtained from the bark of the stem (Mia *et al.*, 2020). Only two species of this genus, tossa jute (*Corchorus olitorius* L.) and white jute (*C. capsularis* L.) are cultivated commercially (Zhang *et al.*, 2019). Both the cultivated species differ in morphological and physiological features. Africa and the Indo-Myanmar regions were identified as the primary and secondary centre of origin, respectively for *C. olitorius*, whereas the *C. capsularis* found to be originated from the Indo-Myanmar region, including South China (Mahapatra *et al.*, 2009). Asian countries produce the majority of the world's jute fibre, with India (56.85%) and Bangladesh (40.67%) accounting for the majority share. West Bengal produces 81% of total jute raw materials in India and accounts for 73% of jute cultivation land (Majumder *et al.*, 2020). Jute fibre is a lignocellulosic fibre that is fully biodegradable, recyclable and environment friendly (Mir *et al.*, 2008). It is primarily cultivated for the production of commercial renewable/biodegradable products such as twines, ropes and sacks (Adeyemo *et al.*, 2021). In addition to fibre, jute leaves are consumed as a leafy vegetables due to their good amount of protein (3.79%), iron (67.93 mg/kg), β -carotene (51.0 mg/kg) and potassium (4400 mg/kg) (Choudhary *et al.*, 2013).

Globally, India is the largest producer and exporter of jute seeds and the demand for quality seeds in the worldwide market

is also increasing over time. As a result, jute breeders must pay close care to the crop for its increased seed production. The elite varieties available are the products of the pure line selection from very few common accessions (Kar *et al.*, 2009). The primary reasons for the recent decrease in jute cultivated area are the narrow genetic base, low seed yield and vulnerability to diseases and pests. The development of a new high-yielding, climate-friendly and agroecological adapted variety was thought to be dependent on genotypic diversity and variability (Bhandari *et al.*, 2017) is primarily based on existing genetic diversity in the population. Diversity can be described as the degree of differentiation between or within species. Existing intra- and inter-specific differences are at the base of all crop improvement programmes. If all the individuals within the species would have been similar, then possibly there could not have been any scope for improvement in plant performances for different traits. Since the beginning of systematic plant breeding, natural variability and divergence between crops have been extensively identified and used in improvement of crop species. However, with the progress of time, natural variability got depleted due to. A precise understanding of the evolutionary connections between germplasm is critical for crop improvement in a various ways, allowing breeders to integrate advantageous variants in their genetic architecture (Naik *et al.*, 2016). Plant breeders frequently assess numerous characteristics in jute crops; however, not all variables can be used as selection criteria for evaluating, characterizing and managing jute germplasm (Maji *et al.*, 2012). In this regard, the first step toward crop improvement will be to characterise the genetic diversity among different germplasm accessions, followed by the use of principal component analysis, a multivariate technique, to explore breeding patterns and eliminate redundancy from large data sets as morphological and physiological variations are common in crop species. The primary objective of PCA analysis is to extract important information, identify it as a set of new orthogonal variables called principal components and map the similarity pattern of observations and variables. While “suitable values” measure the importance and contribution of each component to total variance, each coefficient of appropriate vectors reveals the extent to which each original variable associated with each principal component contributes to total variance (Pachauri *et al.*, 2017). Cluster analysis, on the other hand, is concerned with categorising previously unclassified material. As a result, the current study attempts to evaluate morpho-agronomic seed yield contributing traits as well as genetically diverse genotypes in jute for use in efficient breeding programmes.

Materials and Methods

In this study, 47 accessions of white jute (*C. capsularis*) of diverse geographic origin were used, including 17 exotic (nine from CEX, exploration through NBPGR, New Delhi

and eight from CIJ, Exploration through IJO, Dhaka), 24 indigenous types (CIN), and 6 improved types (CIM) provided by the NAGS, ICAR-CRIJAF, Barrackpore (Table 1). Experiment was conducted in RBD with three replications at the ICAR-Central Seed Research Station for Jute and Allied Fibre at Budbud, West Bengal in 2019-20 and 2020-21 during Rabi season for seed yield and attributing traits. Each genotype was sown in three rows of 3 m length with 40 cm x 10 cm spacing. To obtain a healthy harvest during the season, the necessary agronomical practices were followed. In the context of evaluating germplasm lines for diversity for seed yield related characters, eight quantitative traits (plant height, base diameter, number of primary branches, number of pods, pod length, pod diameter, number of seeds per pod, and seed yield) data was taken on ten selected plants per line.

For quantitative data, descriptive statistics such as mean, minimum and maximum values were estimated before performing an analysis of variance (ANOVA). Quantitative data were normalised before being used as an input for PCA and cluster analysis. Mahalanobis D^2 analysis (1936) was used to compute the distance between genotypes, and the Tochers technique was used to group varieties into clusters, as Rao (1952) explained.

Results and Discussion

The analysis of variance indicated significant differences across genotypes for all eight traits investigated. Based on D^2 values, 47 genotypes were classified into five distinct groups, with genotypes dispersed within each cluster as presented in Figure 1. Cluster I comprised the highest genotypes (43), while clusters II, III, IV, and V each contained only one genotype presented in Table 2.

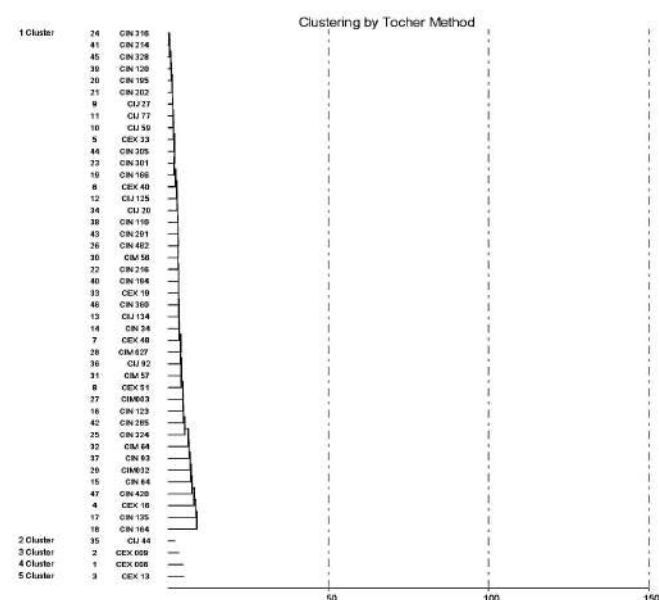


Figure 1: Clustering of 47 white jute genotypes using Tocher method.

Table 1: The list of Capsularis Jute accessions used in this study

S. No.	Accession Name	Country of Origin	IC/EC number	S. No.	Accession Name	Country of Origin	IC/EC number
1.	CEX 006	Indonesia	-	25.	CIN 316	India	IC0549627
2.	CEX 009	Taiwan	EC0502490	26.	CIN 324	India	IC0549633
3.	CEX 13	Brazil	-	27.	CIN 482	India	IC0502222
4.	CEX 16	Australia	-	28.	CIM 003	India	IC0549566
5.	CEX 33	Egypt	EC0592934	29.	CIM 027	India	IC0502827
6.	CEX 40	Burma	-	30.	CIM 032	India	IC0549584
7.	CEX 48	Thailand	-	31.	CIM 56	India	IC0502611
8.	CEX 51	Srilanka	EC0592935	32.	CIM 57	India	IC0502556
9.	CEX 19	Unknown	EC 41336	33.	CIM 64	India	IC0502537
10.	CIJ 27	Thailand	EC0502453	34.	CIJ 20	Thailand	EC0208689
11.	CIJ 59	Nepal	EC 318050	35.	CIJ 44	Thailand	EC0502110
12.	CIJ 77	Pakistan	EC0502459	36.	CIJ 92	USA	EC0502592
13.	CIJ 125	China	EC 345926	37.	CIN 93	India	IC0502317
14.	CIJ 134	China	EC 345913	38.	CIN 110	India	IC0502284
15.	CIN 34	India	IC0502127	39.	CIN 120	India	IC0502187
16.	CIN 64	India	IC0502598	40.	CIN 194	India	IC0549621
17.	CIN 123	India	IC0502086	41.	CIN 214	India	IC0502707
18.	CIN 135	India	IC0502294	42.	CIN 285	India	IC0502258
19.	CIN 164	India	IC0502100	43.	CIN 291	India	IC0502201
20.	CIN 166	India	IC0502198	44.	CIN 305	India	IC0502169
21.	CIN 195	India	IC0502404	45.	CIN 328	India	IC 30730
22.	CIN 202	India	IC0549623	46.	CIN 360	India	IC0502551
23.	CIN 216	India	IC0502166	47.	CIN 420	India	IC 30754/A
24.	CIN 301	India	IC0502276				

Table 2: Clustering pattern of 47 germplasm lines of Capsularis Jute based on D² analysis

S. No.	Clusters	No. of genotypes	Name of genotype
1.	I	43	CIN 316, CIN 214, CIN 328, CIN 120, CIN 195, CIN 202, CIJ 27, CIJ 77, CIJ 59, CEX 33, CIN 305, CIN 301, CIN 166, CEX 40, CIJ 125, CIJ 20, CIN 110, CIN 291, CIN 482, CIM 56, CIN 216, CIN 194, CEX 19, CIN 360, CIJ 134, CIN 34, CEX 48, CIM 027, CIJ 92, CIM 57, CEX 51, CIM 003, CIN 123, CIN 285, CIN 324, CIM 64, CIN 93, CIM 032, CIN 64, CIN 420, CEX 16, CIN 135, CIN 164
2.	II	1	CIJ 44
3.	III	1	CEX 009
4.	IV	1	CEX 006
5.	V	1	CEX 13

Cluster Distance Analysis

Cluster distance analysis revealed that the greatest inter-cluster D² value (66.43) was observed between clusters II and III, followed by cluster IV and II, which had a high D² value of (59.45) highlighted in Table 3. Even though clusters II (Thailand) and V (Brazil) genotypes were originated in different locations they show close relationship since the minimum distance measured between clusters II and V was 14.80. Highest genetic divergence was revealed by Cluster II, with several clusters exhibiting the greatest inter-cluster distances with it, then in Cluster III. Based

on cluster distance analysis a significant amount of intra-cluster genetic divergence also observed among all clusters. Cluster I had the greatest intra-cluster distance (12.96) since it had 43 genotypes, whereas the other clusters only had one genotype. Furthermore, based on D² value clusters that were closest and furthest demonstrated by Figure 2. Starting a plant breeding programme obliges genetic study of quantitative characteristics, which may lead to a systemic approach of design as well as proper planning of plant breeding strategies. D² analysis is a reliable method for estimating the divergence present in the plant population. It

Table 3: Average intra and inter cluster D² values

	I	II	III	IV	V
I	12.96	33.09	25.26	25.56	36.19
II		0.00	66.43	59.45	14.80
III			0.00	31.78	48.57
IV				0.00	47.32
V					0.00

assesses differentiation at the inter-cluster and within-cluster levels and aids in the selection of genetically divergent parents for use in the hybridization procedure based on higher average performance. Clusters II and III exhibited the highest divergence based on D² values, followed by clusters IV and II. While, clusters II and V have the least genetic difference or the closest relationship. Furthermore, it was discovered that cluster II was the most diverse among of the five clusters, followed by cluster III. Similarly, cluster I had the highest intra-cluster distance since it had 43 genotypes, whereas the other clusters only had one genotype each. Henceforth, choosing genotypes from clusters with a higher inter-cluster distance is appropriate (clusters II and III).

Cluster Mean Analysis

Similarly, a cluster mean analysis of eight yield attribute characteristics was performed and the results are shown in Table 4. The mean comparison of the various characters revealed significant differences among the clusters for all the characters examined. Cluster II had the highest mean plant height (194.70 cm), followed by cluster I (152.76), and cluster III had the lowest mean plant height (93.30 cm). cluster I had the highest mean for base diameter (13.30), followed by Cluster IV (10.83), while cluster III had the lowest mean (7.75). Cluster II had the highest mean for the number of primary branches per plant (6.75), followed by cluster V (5.70), and Cluster I had the lowest mean of 4.70. cluster II had the greatest mean for the number of pods per plant (64.50), followed by cluster I (41.42), while cluster III had the lowest mean of 26.80. cluster V had the highest mean pod length of 12.45, followed by III at 11.07, while cluster II had the lowest mean of 9.75. Cluster V had the highest mean pod

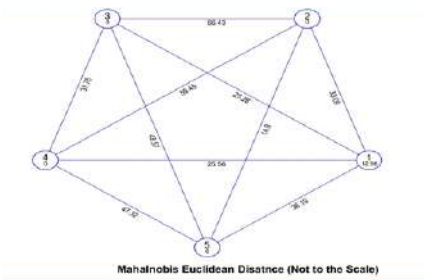


Figure 2: Intra and inter-cluster distances among five clusters of white jute genotypes.

diameter of 12.55, followed by cluster IV at 12.32, and cluster III had the lowest mean of 9.62. Cluster IV had the greatest mean of 49.50 seeds per pod, followed by cluster III (38.50), while cluster II had the lowest mean of 31.85 seeds per pod. Seed yield recorded a maximum mean in cluster II of 31.05 followed by V of 25.65 while the minimum mean of 10.94 was observed in cluster III. Similar results on genetic divergence were also reported by many other researchers in Tossa Jute (*C. oltorius*) (Debnath *et al.*, 2018 with Biswas *et al.*, 2018).

All 47 genotypes were distributed over five clusters, and averages for all eight characteristics were calculated across the clusters, as shown in Table 4. As a result, the first rank assigned based on highest cluster mean value and the following cluster with the next best means was given the second, third, and so on up to the fifth rank for all characteristics. Finally, the clusters are sorted using the total score derived from the eight characters. The cluster with the lowest score was given first place, and the cluster with a higher score than the preceding one was given second, third, and so on up to fifth place. As a result, cluster II took first place with an overall score of 18 across the eight characteristics, followed by clusters V, IV, I, and III, suggesting the presence of the most promising genotypes in them and the possibility of future breeding efforts to generate new material.

Cluster analysis is a statistical approach for identifying groups or clusters of comparable properties in multidimensional space based on item similarity. This work uses cluster analysis to identify genotypes with high seed yield and their corresponding contributing characteristics.

Table 4: Cluster mean analysis

S. No.	Clusters	No. of genotypes	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	Cluster Score	Cluster rank
1	I	43	152.76 (2)	9.39 (3)	4.70 (5)	41.42 (2)	10.08 (4)	9.95 (3)	33.04 (4)	16.46 (3)	26	IV
2	II	1	194.70 (1)	13.30 (1)	6.75 (1)	64.50 (1)	9.75 (5)	9.95 (3)	31.85 (5)	31.05 (1)	18	I
3	III	1	93.30 (5)	7.75 (5)	5.15 (4)	26.80 (5)	11.07 (2)	9.62 (4)	38.50 (2)	10.94 (5)	32	V
4	IV	1	132 (3)	10.83 (2)	5.20 (3)	27.48 (4)	11.05 (3)	12.32 (2)	49.50 (1)	11.18 (4)	22	III
5	V	1	109.20 (4)	9.30 (4)	5.70 (2)	38 (3)	12.45 (1)	12.55 (1)	36.50 (3)	25.65 (2)	20	II

Where,

X₁ = Plant Height, X₂ = Base Diameter, X₃ = Number of primary branches per plant, X₄ = Number of pods per plant
X₅ = Pod Length, X₆ = Pod Diameter, X₇ = Number of seeds per pod, X₈ = Seed Yield

Based on cluster means, a wide range of genetic variation for seed yield and its contributing characteristics was discovered in white jute genotypes. Similarly, choosing genotypes with multiple desired characteristics based on cluster mean is always viable. As a result of the cluster analysis and PCA, 47 germplasm lines of white jute were classified into five groups. A similar sort of grouping pattern for 90 jute genotypes using hierarchical cluster analysis and PCA was also analysed (Ngomuo *et al.*, 2017). In the current investigation, the Euclidian distance revealed that clusters were typically formed depending on the geographical region of the genotypes. It was also observed that eight jute genotypes divided into three groups based on seven morphological characteristics (Mukul *et al.*, 2020). In case of white jute 15 white jute genotypes divided into four groups based on four fibre yield-related characteristics (Md. Mia *et al.*, 2020).

Contribution of Eight Morphological Characters Towards Diversity

In this study we also noticed the percent contribution of eight morphological characteristics to diversity in this study (Figure 3). Seed yield contributed the most to diversity (47.27%) of the eight characters studied, followed by pod length (12.21%), pod diameter (11.66%), number of pods per plant (9.07%), number of seeds per pod (8.51%), number of primary branches per plant (5.74%), and plant height (5.37%). Whereas, the contribution of base diameter to divergence is the lowest (0.19%). Furthermore, germplasm line CIJ-44 had the highest seed yield of 31.05 g per plant, as well as the highest plant height of 194.70 cm, base diameter of 13.30 mm, number of primary branches per plant 6.75, number of pods per plant 64.5, pod length 9.75 cm, pod diameter of 9.95 mm, and number of seeds per pod 31.85. In comparison, the CEX-13 germplasm line had the maximum seed yield of 25.65 g per plant, followed by CIN-324 (23.95 g), CIN-420 (23.55 g), and CIN-123 (23.55 g). This study revealed that seed yield contributed the maximum towards genetic diversity

among all eight characters studied followed by pod length. Similarly, Tossa Jute genotypes reported that the number of pods per plant contributed maximum towards genetic divergence, followed by seed yield per plant (Das *et al.*, 2012).

Principal Component Analysis

Principal components analysis of white jute genotype revealed that the first four principal components with eigenvalues more than 1.00 contributed 78.09% to the variance in the data. PC1 contributed 28.43% of the overall variation, with large loadings in plant height (0.48) and pod length (0.43). The PC2 expressed 19.76% of the variation, with large contributions from pod diameter (0.50) and plant height (0.39). The combined contribution of PC1 and PC2 to the variance was 48.19%. The PC3 accounted for 16.36% of the overall variation, with the number of pods per plant playing a significant role (0.37) highlighted in Table 5. While, PC4 expressed 13.54% of the overall variation, with the number of primary branches per plant (0.71) significantly contributed.

Principal component analysis (PCA) is a technique for identifying the characteristics that contribute to the primary sources of variation in a germplasm panel and genetic diversity study. It finds the characteristics that contribute the most to the most observed variance within a broad set of genotypes and is used to minimise data complexity while decreasing variation and enhancing interpretability. It explains how genotypes in similar categories group

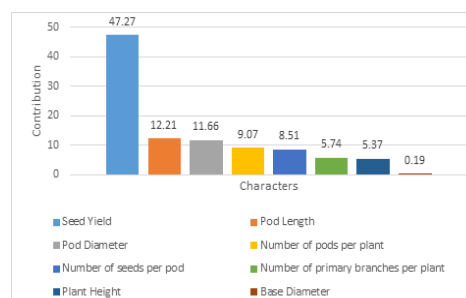


Figure 3: Percent contribution of 8 characters towards diversity in white jute genotypes

Table 5: Principal components analysis of 47 white jute accessions studied based on eight quantitative traits.

Trait	PC1	PC2	PC3	PC4
Plant height	0.48	0.39	0.26	0.08
Base diameter	0.22	-0.61	-0.05	-0.19
Number of primary branches per plant	0.09	-0.26	0.35	0.71
Number of pods per plant	-0.48	0.14	0.37	-0.38
Pod length	0.43	-0.35	-0.20	-0.20
Pod diameter	0.42	0.50	-0.13	0.01
Number of seeds per pod	-0.10	0.12	-0.71	0.03
Seed yield	0.32	-0.03	0.32	-0.51
Eigen value (root)	2.27	1.58	1.31	1.08
%Variance explained	28.43	19.76	16.36	13.54
Cumulative variance explained	28.43	48.19	64.56	78.09

together against those in different categories. The variance explained by each principal component is indicated by the eigen values in PCA and decreases monotonically from the first to the final principal component. In our experiment, the first four principal components accounted for 78.09% of the overall variance, indicating a high correlation between all of the characteristics being investigated. The first PC was the most significant, that exclusively accounted for 28.43% of the total variance. As a result of their large loadings, plant height and pod length characteristics were crucial in differentiating the genotypes in the first PC. Similarly, the first and second PCs to explain 85.72% of the overall variability (Sawarkar *et al.*, 2015). Genetic diversity is a key deciding factor while choosing of parents. Furthermore, our research gives critical information to jute breeders, assisting them in optimising the selection of plant material to be employed in breeding programmes for this vital natural fibre crop.

In conclusion, the present study described that 47 genotypes clustered into five different groups based on genetic divergence of eight seed yield attributed traits. The highest contribution in genetic divergence is done by seed yield followed by pod length and diameter. Among all 47 germplasm lines, CIJ-44 showed the most significant response for seed yield attributed traits followed by CEX-13, CIN-324 CIN-420 and CIN-123. Principle component analysis revealed that highest contribution in variation exerted by PC1 along with large loadings in plant height and pod length. Hence, genetic diversity analysis provide an opportunity to distinguish the genotypes at inter or intra cluster level and help identify genetically diverse parent for hybridization programme. Furthermore, our research gives critical information to jute breeders, assisting them in optimising the selection of plant material to be employed in breeding programmes for this valuable natural fibre crop.

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Conflict of Interest

Nil

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RESEARCH ARTICLE

Biochemical and Physiological Factors Imparting Tolerance in Safflower against Aphid, *Uroleucon compositae* (Theobald)

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Abstract

Development of aphid tolerant cultivars is needed in pest management in safflower, where the crop is often grown with least plant protection measures. Sixteen recombination inbred lines (RILs) of F₇ generation of the cross, CO-1 (susceptible) X EC-523368-2 (tolerant) along with parents were studied to understand the biochemical and physiological factors operating in tolerant RILs. Eight RILs were confirmed tolerant (A.I.I., 1.1 - 1.5) and 8 RILs were found highly susceptible (A.I.I., 4.5- 5.0) to aphid. Susceptible RILs underwent more oxidative stress through more H₂O₂ (4908.0 ± 1287 nmols g⁻¹ fresh weight) production due to aphid infestation compared to tolerant RILs. Activity reactive oxygen species (ROS) enzymes, superoxide dismutase (53.63 ± 0.29 (nmols g⁻¹ fresh weight) and catalase (33.73 ± 3.3 μmols g⁻¹ fresh weight) and amount of metabolites like total phenols (169.60 ± 16.49 μg g⁻¹ gallic acid equivalent) were more intolerant RILs than susceptible ones. The tolerant RILs were physiologically more efficient with higher chlorophyll (8.45 ± 0.91 mgL⁻¹), net photosynthesis (28.9 ± 3.31 μmole CO₂ m⁻² sec⁻¹), net assimilation rate (0.289 ± 0.033 μmole CO₂ cm⁻² leaf area) and intrinsic water use efficiency (0.35 ± 0.05 μmole CO₂ mole⁻¹ H₂O) than the susceptible RILs.

Keywords: Aphid, Biochemical factors, Physiological factors, Safflower, Tolerance, *Uroleucon compositae*.

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Introduction

Safflower (*Carthamus tinctorius* L.) is an important oilseed crop, grown in India since ages for its high-quality edible oil. This is cultivated on residual soil moisture in *Rabi* (winter) season. Safflower is mainly cultivated in the states of Maharashtra, Karnataka, Telangana, Madhya Pradesh, Gujarat and parts of Andhra Pradesh under different cropping systems. India's total safflower production is 1.22 MT from an area of 1.56 lakh ha with a productivity of 782 kg per ha (Mukta *et al.*, 2017). Aphid *Uroleucon compositae* (Theobald) is considered as a major pest. A yield loss up to 78.5% was recorded on susceptible variety compared to a 48.5% yield loss on moderately tolerant when proper control measures were not taken (IIOR, 2015). Both nymphs and adults suck sap from a shoot and young leaves, due to which the plant growth is stunted. In case of a severe attack of the aphid, the plants start showing yellowing and drying, resulting in premature death of plants. In addition, aphid also excretes honeydew, which falls on the upper surface of below leaves on which sooty mold develops, hindering the photosynthetic activity (Balikai, 2000). Mostly, the safflower crop is grown by small and marginal farmers with low inputs and may not receive any plant protection measures many times (Hanumantharaya *et al.*, 2007) to avoid production cost. This often results in to significant yield loss. Many authors have studied

the reaction of safflower to aphids under natural infestation (Singh, 2008; Rajput *et al.*, 2013; Guljar and Rajesh, 2016). Few safflower accessions were reported for their reaction under the artificial release of aphids (Srinivas and Mukta, 2015; Mukta *et al.*, 2017).

A stay green safflower germplasm line, EC-523368-2 has been identified, which showed higher levels of tolerance to aphid (DOR, 2012). The stay green character indicated that this tolerant genotype is physiologically more efficient than other genotypes. Tolerance is distinctive in terms of the plant's ability to withstand or recover from herbivore injury through growth and compensatory physiological processes. The tolerant plants can compensate photosynthetically by avoiding feedback inhibition and impaired electron flow through photosystem II resulting from insect feeding. Similarly, the up-regulation of peroxidases and other oxidative enzymes during insect feeding, in conjunction with elevated levels of phytohormones, can play an important role in providing plant tolerance to insect pests (Kyle *et al.*, 2016).

However, very little information is available on such mechanisms of tolerance in safflower against aphid. Therefore, the present investigation is undertaken to analyze the physiological and biochemical factors imparting tolerance in safflower to aphids.

Materials and Methods

The present study was carried out at ICAR- IIOR Farm, ICRISAT (17.530° N Latitude and 78.270° E Longitude) during *Rabi* season of 2018-2019. In previous years, a germplasm accession, EC-523368-2 was identified as stay green and highly tolerant to aphid. Based on the reaction of around 300 RILs of the cross, CO-1 X EC-523368-2 evaluated in the F₆ generation in the previous year, 8 tolerant and 8 susceptible recombination inbred lines (RILs) were selected. The same set of 16 RILs of F₇ generation and their parents were evaluated during *Rabi*, 2018, to confirm their reaction to aphid.

All 16 RILs along with parents (also checks) were sown on 14th December 2018 in two replications following a completely randomized block design. Each RIL was raised in 3 rows of 2 m in length each with a spacing of 45 x 10 cm. Infester plants of susceptible variety, CO-1 was sown, one month before sowing of test entries a separate block away from the main screening block. When the test entries reached stem elongation stage (~ 40 day old), infester plants with aphids were cut and distributed @ 1 plant per 1 m row (IIOR, 2018). Aphids were moved to the test entries, multiplied and caused damage symptoms. Five plants from each RIL were randomly selected in each replication and when susceptible check, CO-1 was completely got killed, the injury rating was given on a 1-5 scale based on % yellowing and drying of foliage viz., 0 to 20% - 1; 21 to 40% - 2; 41 to

60% - 3; 61 to 80% - 4 and 81 to 100% - 5 and aphid infestation index (A.I.I.) was calculated by using the following formula:

$$A.I.I. = \frac{1 \times a + 2 \times b + 3 \times c + 4 \times d + 5 \times e}{a + b + c + d + e}$$

Where, a, b, c, d and e are the actual number of plants falling in each of the 5 corresponding foliage drying grades *i.e.*, 1 to 5. Finally, the mean of A.I.I. was calculated and the entries were classified into different grades as - highly tolerant (A.I.I., 1.0), tolerant (A.I.I., >1.0 to 2.0), moderately tolerant (A.I.I., >2.0 to 3.0), susceptible (A.I.I., >3.0 to 4.0) and highly susceptible (A.I.I., >4.0 to 5.0).

Same set of 16 RILs were also raised in a separate block aphid as free regime that was away from main screening block to avoid migration of aphid. Recommended systemic insecticide was sprayed regularly to free the plants from aphids.

After 10 days of aphid release (DAAR), plants showed symptoms due to feeding of aphids. Top 5 cm twig portion of the plants from both aphid-free and aphid-infested plants from each replication were cut with a fine blade and the samples were brought to the laboratory and stored at -20°C in deep freezer till the plant analysis was done.

Lipid Peroxidation, Antioxidative Enzymes and Metabolites

Preparation of Various Extracts

Unless stated otherwise, all extraction procedures were carried out at 0 to 4°C. Each experiment was repeated thrice and the estimations further made in duplicate. Preliminary experiments were conducted to optimize the extraction conditions with respect to pH, molarity and type of buffer, the concentration of stabilizing agent(s) and other constituents of the extraction medium. Finally, the standardized extraction medium for superoxide dismutase (SOD) and peroxidase (POX) consisted of 0.1 M Tris-HCl buffer (pH 7.5) containing 3% (w/v) polyvinylpyrrolidone, 1 mM EDTA and 1mM CaCl₂. The extraction medium for Catalase (CAT) and ascorbate peroxidase (APX) consisted of 0.1 M potassium phosphate buffer (pH 7.5) in place of Tris-HCl buffer, the rest extractants being the same.

The enzymes were extracted by macerating 2 g tissue with 7.2 mL of ice-cold extraction medium in a pre-chilled pestle and mortar placing in ice bath. The homogenate was filtered through four-layered muslin cloth and the filtrate was centrifuged at 15,000 rpm for 20 minutes in a refrigerated centrifuge at 4°C. The supernatant (7.3 mL) was carefully decanted, labelled and stored in deep freezer at -20°C. Trichloro acetic acid (TCA) extract was prepared and used for the estimation of malondialdehyde (MDA) and total glutathione. One gram of tissue was ground with 5 mL of 0.1% TCA. The extract was filtered through four-layered muslin cloth and centrifuged at 12,000 rpm for 15 minutes at 4°C. The supernatant was collected and used for the analysis.

Lipid Peroxidation

MDA, the level of lipid peroxidation was measured in terms of MDA by using 2- thiobarbituric acid (TBA) reaction employing slightly modified method of Heath and Packer (1968). The MDA concentration was calculated using the molar extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ (Dipierro and Leonardis, 1997) and expressed as nmol of MDA g^{-1} fresh weight. Hydrogen peroxide (H_2O_2), an important reactive oxygen species (ROS) and its higher concentration in the cell, causes lipid peroxidation. The amount of H_2O_2 was estimated by Sinha (1972) method and expressed as nmol of H_2O_2 g^{-1} fresh weight.

Antioxidative Enzymes

SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) (Beauchamp and Fridovich, 1971). Percent inhibition was calculated by the following formula of Asada *et al.* (1974). Percent inhibition = $[(V - v)/V] \times 100$, Where, V = rate of assay reaction in absence of SOD, v = rate of assay reaction in presence of SOD. The amount of SOD was expressed as nmol g^{-1} fresh weight. Catalase activity was measured by slightly modified method of Sinha (1972) and the amount of CAT expressed as $\mu\text{mol g}^{-1}$ fresh weight. Peroxidase was assayed by determining the rate of Guaiacol oxidation in the presence of H_2O_2 at 470 nm (Rao *et al.*, 1996). Ascorbate peroxidase was assayed by the method of Nakano and Asada (1981). The amount of POX and APX was expressed as nmols $\text{min}^{-1} \text{ g}^{-1}$ fresh weight.

Metabolites

Total phenols quantity was determined by the method of Ainsworth and Gillespie (2007) and expressed as $\mu\text{g g}^{-1}$ gallic acid equivalent (GAE). Total glutathione content was estimated by the method suggested by Smith (1985) and expressed as nmols g^{-1} fresh weight.

Physiological Factors

Total chlorophyll content (Chlorophyll 'a' and 'b') was estimated from the leaf samples collected from safflower RILs at 10 DAAR, by using the method described by Arnon

(1949) and expressed as mg L^{-1} . Net photosynthesis (P_n), net assimilation rate (NAR) and intrinsic water use efficiency (iWUE) were calculated by measuring amount of CO_2 and water vapor exchange in attached leaves through a portable gas exchange measuring system (Model LI-6400, LI-COR, USA) (Ratnakumar *et al.*, 2013) and expressed as $\mu\text{mole CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$, $\mu\text{mole CO}_2 \text{ cm}^{-2}$ leaf area and $\mu\text{mole CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$, respectively.

Statistical Analysis

The data pertaining to all tolerant RILs and susceptible RILs were pooled separately replication-wise. The data were analyzed using the analysis of variation (ANOVA) using SPSS software. Means were separated by LSD at 5% level of significance.

Results and Discussion

There was significant differential reaction showed by safflower RILs to aphids. Out of 16 RILs evaluated, 8 RILs were found tolerant and 8 RILs were found highly susceptible to aphid. The tolerant check, EC-523368-2 and susceptible check, CO-1 was recorded an average A.I.I of 1.3 (tolerant) and the highest A.I.I of 5.0 (highly susceptible), respectively (Table 1).

Lipid Peroxidation (MDA, H_2O_2)

Both MDA and H_2O_2 content indicate the extent of lipid peroxidation in the plants. The MDA content was significantly more in susceptible lines (2.60 ± 0.19 nmols g^{-1} fresh weight) compared to tolerant lines after 10 days of aphid infestation. When tolerant lines were attacked by aphids MDA content was increased by 27.0%. Susceptible lines produced 9.24% more MDA compared to tolerant lines (t-test, $p = 0.04$) when challenged with aphid infestation. With aphid infestation, H_2O_2 content has significantly increased in both tolerant and susceptible lines by 19.0 and 12.25%, respectively compared to aphid free condition. Susceptible lines produced 10.27% more H_2O_2 than tolerant lines (Table 2). Increased lipid peroxidation was observed in both tolerant and susceptible lines whenever plants underwent stress

Table 1: Reaction of safflower RILs to aphid, *U. compositae* during Rabi 2018-19

RILs	A.I.I.	Category	RILs	A.I.I.	Category
RIL-6	1.1	Tolerant	RIL-81	5.0	Highly susceptible
RIL-14	1.1	Tolerant	RIL-114	5.0	Highly susceptible
RIL-18	1.1	Tolerant	RIL-152	5.0	Highly susceptible
RIL-34	1.2	Tolerant	RIL-201	5.0	Highly susceptible
RIL-218	1.3	Tolerant	RIL-210	5.0	Highly susceptible
RIL-222	1.2	Tolerant	RIL-250	4.5	Highly susceptible
RIL-235	1.5	Tolerant	RIL-322	5.0	Highly susceptible
RIL-351	1.2	Tolerant	RIL-358	5.0	Highly susceptible
EC-523368-2	1.3	Tolerant	CO-1	5.0	Highly susceptible

RIL- Recombinant Inbred Line, A.I.I- Aphid Infestation Index

Table 2: Lipid peroxidation in tolerant vs susceptible RILs to aphid in safflower

Nature of RILs	Mean + SEM		p<=0.05	% Change	ANOVA			
	Aphid free	Aphid infested			Source	F	df	p<=0.05
Malondialdehyde (MDA) (nmols g ⁻¹ fresh weight)								
Tolerant	1.87 + 0.14	2.38 + 0.25	0.05	27.0	Tolerance	50.6	17,35	<0.0001
					Aphid infestation	2854	1,35	<0.0001
Susceptible	1.94 + 0.15	2.60 + 0.19	0.04	34.0	Tolerance x Aphid infestation	33.5	17,35	<0.0001
Hydrogen peroxide (H ₂ O ₂) (nmols g ⁻¹ fresh weight)								
Tolerant	3725 + 640	4435 + 1035	0.0008	19.0	Tolerance	37.3	17,35	<0.0001
					Aphid infestation	119	1,35	<0.0001
Susceptible	4372 + 850	4908 + 1287	0.004	12.25	Tolerance x Aphid infestation	36.6	17,35	<0.0001

Table 3: Activity of ROS enzymes in tolerant vs susceptible RILs to aphids in safflower

Nature of RILs	Mean + SEm		p <=0.05	% Change	ANOVA			
	Aphid free	Aphid Infested			Source	F	df	p<=0.05
Superoxide Dismutase (SOD) (nmols g ⁻¹ fresh weight)								
Tolerant	52.79 + 0.35	53.63 + 0.29	0.002	1.6	Tolerance	318	17,35	<0.0001
					Aphid infestation	792	1,35	<0.0001
Susceptible	49.58 + 0.31	43.29 + 0.64	0.004	12.7	Tolerance x Aphid infestation	126	17,35	<0.0001
Catalase (CAT) (μmols g ⁻¹ fresh weight)								
Tolerant	28.93+3.82	33.73+3.3	0.001	16.59	Tolerance	40.46	17,35	<0.0001
					Aphid infestation	130.19	1,35	<0.0001
Susceptible	18.72 + 2.73	23.66+1.96	0.00001	26.38	Tolerance x Aphid infestation	2.95	17,35	<0.003
Peroxidase (POX) (nmols min ⁻¹ g ⁻¹ fresh weight)								
Tolerant	92.77+9.5	163.36+38.0	0.0009	175.0	Tolerance	2664	17,35	<0.0001
					Aphid infestation	85127	1,35	<0.0001
Susceptible	107.8+21.6	328.25+66.46	<0.0001	204.5	Tolerance x Aphid infestation	2102	17,35	<0.0001
Ascorbate Peroxidase (APX) (nmols min ⁻¹ g ⁻¹ fresh weight)								
Tolerant	112.98+33.9	721.67+179.6	<0.0001	538.7	Tolerance	121	17,35	<0.0001
					Aphid infestation	21071	1,35	<0.0001
Susceptible	144.48+36.58	633.67+132	<0.0001	339.5	Tolerance x Aphid infestation	109	17,35	<0.0001

due to aphid infestation but was more in susceptible lines than the tolerant lines. Lipid peroxidation has been often assessed by monitoring the changes in MDA value (Mondal *et al.*, 2006). Increase in peroxidation of membrane lipids during oxidative stress is due to more production of reactive oxygen species (ROS) (Wise, 1995). Hydrogen peroxide is one of the ROS capable of causing oxidative damage besides other species like, superoxide radical (O₂⁻), perhydroxy radical (HO₂⁻), hydroxyl radical (OH⁻), peroxy radical (ROO⁻) and singlet oxygen (O₂) (Krieger-Liszkay, 2005). These ROS react with various cellular targets resulting in DNA and protein damage and in lipid peroxidation (Apel and Hirt, 2004).

Antioxidative Enzymes (SOD, CAT, POX and APX)

The SOD activity in tolerant lines with a mean value of 52.79 nmols g⁻¹ fresh weight under aphid free condition increased to mean value of 53.63 nmols g⁻¹ fresh weight with aphid infestation. In susceptible lines, SOD reduced by

12.7% (43.29 nmols g⁻¹ fresh weight) after aphid infestation. SOD was lesser in susceptible lines by 19.3% compared to tolerant lines (t-test, p = 0.002) (Table 3). SOD is the first line of defense against oxyradical-mediated injury (Van Camp *et al.*, 1996). It catalyzes the dismutation of O₂⁻ into H₂O₂ and plays an important role in protecting cells against superoxide derived oxidative damage (Rabinowitch and Fridovich, 1983) in plants (Giannopolitis and Ries, 1977). The increase in SOD activity was reported in wheat due to *D. noxia* feeding (Ni and Quisenberry, 2003) and in cassava due to *Tetranychus cinnabarinus* (Lu *et al.*, 2017).

Catalase is one of the primary enzymatic defenses against oxidative stress (Zimmermann *et al.*, 2006). In the present study, activity of CAT was increased in tolerant lines with a mean value of 33.73 μmols g⁻¹ fresh weight than in susceptible lines (23.66 μmols g⁻¹ fresh weight) after aphid infestation (t-test, p = 0.001) (Table 3). CAT activity was 29.84% higher in tolerant lines than the susceptible

lines after aphid infestation. This showed that catalase effectively scavenged H_2O_2 that was produced due to aphid infestation in tolerant lines compared to susceptible lines. Most of the catalase activity is associated with peroxisomes where it removes the hydrogen peroxide formed during photorespiration. Therefore, in plants, CAT is often considered to be a peroxisomal marker enzyme because of its presence in these organelles (Corpas *et al.*, 1993). Higher activity of CAT was reported in resistant black gram genotypes than in susceptible genotypes against whiteflies, *Bemisia tabaci* (Kumar *et al.*, 2012). CAT activity was positively related to resistance in transgenic cassava lines to *T. cinnabarinus* (Lu *et al.*, 2017).

Peroxidases are another group of non-chloroplastic enzymes that detoxify H_2O_2 in the cytosolic part of cell. POX appeared to be a major antioxidative enzyme in safflower against aphid. When challenged with aphids, its activity was increased by 175.0 and 204.5% in tolerant and susceptible lines. With aphid infestation, POX activity was doubled ($328.25 \text{ nmols min}^{-1} \text{ g}^{-1}$ fresh weight) in susceptible lines compared to tolerant lines ($163.36 \text{ nmols min}^{-1} \text{ g}^{-1}$ fresh weight) (Table 3). POX would be involved in the scavenging of H_2O_2 that is not removed by CAT (Willekens *et al.*, 1997). Aphid infestation induced POX activity in all cultivars of wheat against *Sitobion avenae*, especially in susceptible ones Han *et al.* (2009).

Aphid infestation strongly induced the positive activity of APX aphid-infested plants in both tolerant and susceptible plants. With aphid feeding, APX activity was increased by 538.7% ($721.67 \text{ nmols min}^{-1} \text{ g}^{-1}$ fresh weight) in tolerant lines and 339.5% ($633.67 \text{ nmols min}^{-1} \text{ g}^{-1}$ fresh weight) in susceptible lines. Tolerant lines had 12.19% more APX activity than the susceptible lines when infested with aphids (Table 3). Ascorbate peroxidase is another important enzyme which plays a pivotal role in eliminating H_2O_2 from plant cells. Primary purpose of APX is to scavenge H_2O_2 before it can react with cellular biomolecules and cause damage (Shigeoka *et al.*, 2002). Aphid-induced APX activity to a higher level in a less susceptible cultivar of triticale than in a more susceptible one against *Sitobion avenae* (Iwona *et al.*, 2012). The oligophagous species, *Rhopalosiphum padi* caused stronger induction of APX activity in tested triticale than the monophagous species *S. avenae*.

Metabolites (Total phenols and Glutathione)

With aphid infestation total phenol content in safflower plants was increased in both tolerant and susceptible lines by 8.04 and 5.83%, respectively. Total phenols were more in tolerant lines ($169.60 \mu\text{g g}^{-1}$ gallic acid equivalent) than susceptible lines ($157.00 \mu\text{g g}^{-1}$ gallic acid equivalent) after aphid infestation. In the presence of aphid infestation, tolerant lines produced 8.02% more phenols than that of susceptible lines (t-test, $p = 0.01$) (Table 4). The majority of

plant phenolic compounds are toxic to herbivorous insects, including aphids and impair their growth, development and fecundity (Dreyer and Campbell, 1987). An increase in total phenols with aphid infestation was earlier reported in mustard (Sharma and Rao, 2013) and cotton (Divya *et al.*, 2017). Negative correlation between the total phenols and mustard aphid, *L. erysimi* population was reported by Ram *et al.* (1995) and Jat *et al.* (2007).

Aphid infestation induced total glutathione in susceptible and tolerant lines by 69.0% more than aphid free lines. The glutathione content has been significantly increased in susceptible lines (t-test, $p = 0.0001$) that were infested with aphid ($11497.0 \text{ nmols g}^{-1}$ fresh weight) than tolerant lines ($10030.0 \text{ nmols g}^{-1}$ fresh weight) (Table 4). It indicated that glutathione increased with an infestation of aphids in order to scavenge ROS produced in both tolerant and susceptible plants. Glutathione is an antioxidant also plays a role in scavenging active oxygen species (Dhindsa, 1987; Noctor and Foyer, 1998).

Physiological Factors (Total chlorophyll, P_n , NAR and iWUE)

The impact of aphid feeding on physiological factors viz. total chlorophyll, net photosynthesis, net assimilation rate and intrinsic water use efficiency in tolerant and susceptible safflower lines was studied. Total chlorophyll content was reduced with an infestation of aphids in both the tolerant and susceptible lines. However, the loss of chlorophyll in susceptible lines was significantly more (33.0%) while tolerant lines lost only 11.0% of chlorophyll due to aphid feeding. Susceptible lines had 21.35% less chlorophyll than that of tolerant lines (t-test, $p = 0.01$) (Table 5). Chlorophyll levels change during plant development (Costa *et al.*, 2001), and can alter in response to a wide variety of stresses (Lawson *et al.*, 2001). Chlorophyll content can be reduced by insect feeding, nutritional deficiencies and pathogen infections (Ni *et al.*, 2002). Significant chlorophyll loss in infested plants was reported in *Pisum sativum* L., *Vicia faba* L., *Trifolium pretense* L., *Medicago sativa* L. due to feeding by *Acyrtosiphon pisum* (Sylwia *et al.*, 2010) and in maize by *R. padi* and *S. avenae* (Hubert *et al.*, 2013). Loss of chlorophyll is 40% in the susceptible soybean due to aphid, *Aphis glucines* (John *et al.*, 2007).

In susceptible lines, net photosynthesis (P_n) was reduced by 32.0% due to aphid infestation while it is only 7.7% reduction in tolerant lines when compared to respective lines in aphid free conditions. Because of aphid infestation, net photosynthesis was reduced by 36.0% in susceptible lines compared to tolerant lines. Net assimilation rate (NAR) also followed the same trend as that of net photosynthesis. This clearly showed that the tolerant lines were more efficient photosynthetically than that of susceptible lines. This may be why the tolerant lines stay green even after aphid infestation.

Table 4: Plant metabolites in tolerant vs susceptible RILs to aphids in safflower

Nature of RILs	Mean + SEM		p<=0.05	% Change	ANOVA			
	Aphid free	Aphid Infested			Source	F	df	p<=0.05
Total phenols (µg g ⁻¹ Gallic Acid Equivalent)								
Tolerant	156.97+26.3	169.60+16.49	0.04	8.04	Tolerance	61.8	17,35	<0.0001
Susceptible	157.00+11.9	147.84+20.84	0.01	5.83	Aphid infestation	3.1	1,35	0.0891
					Tolerance x Aphid infestation	36.5	17,35	<0.0001
Total Glutathione (nmols g ⁻¹ fresh weight)								
Tolerant	5960+1256	10030+1896	<0.0001	69.0	Tolerant	7075	17,35	<0.0001
Susceptible	6825+1194	11497+1287	<0.0001	68.4	Aphid infestation	44491	1,35	<0.0001
					Tolerance x Aphid infestation	6445	17,35	<0.0001

Table 5: Comparison of different physiological factors in tolerant vs susceptible RILs to aphids in safflower

Nature of RILs	Mean + SEm		p<=0.05	% Change	ANOVA			
	Aphid free	Aphid Infested			Source	F	df	p<=0.05
Total chlorophyll (mg L ⁻¹)								
Tolerant	9.92 + 0.6	8.45 + 0.91	0.03	11.0	Tolerance	20.2	17,35	<0.0001
Susceptible	9.88 + 0.78	6.65 + 1.34	0.01	33.0	Aphid infestation	563	1,35	<0.0001
					Tolerance x Aphid infestation	9.46	17,35	<0.0001
Net Photosynthesis (Pn) (μmole CO ₂ m ⁻² sec ⁻¹)								
Tolerant	31.32 + 3.09	28.9 + 3.31	0.085	7.7	Tolerant	16.74	17,35	<0.0001
Susceptible	27.06 + 2.62	18.5 + 0.07	0.023	32.0	Aphid infestation	81.33	1,35	<0.0001
					Tolerance x Aphid infestation	3.74	17,35	0.0005
Net Assimilation Rate (NAR) (μmole CO ₂ cm ⁻² leaf area)								
Tolerant	0.31 + 0.03	0.289 + 0.033	0.05	6.7	Tolerant	16.74	17,35	<0.0001
Susceptible	0.27 + 0.026	0.185 + 0.07	0.01	31.0	Aphid infestation	81.33	1,35	<0.0001
					Tolerance x Aphid infestation	3.74	17,35	0.0005
Intrinsic Water Use Efficiency (iWUE) (μmole CO ₂ mol ⁻¹ H ₂ O)								
Tolerant	0.41 + 0.05	0.35 + 0.05	0.018	14.6	Tolerant	16.74	17,35	<0.0001
Susceptible	0.34 + 0.05	0.16 + 0.07	0.05	52.9	Aphid infestation	81.33	1,35	<0.0001
					Tolerance x Aphid infestation	3.74	17,35	0.0005

Aphid-tolerant lines have more intrinsic water use efficiency (iWUE) than susceptible lines. Aphid infestation reduced iWUE of tolerant lines by 14.6 while it was 52.9% in case of susceptible lines. The study clearly showed that the tolerant lines are more efficient in water usage ($0.35 \mu\text{mole CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) compared to susceptible lines ($0.16 \mu\text{mole CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) when under stress of aphid infestation. Aldea *et al.* (2005) reported that herbivorous insects will cause water loss in the infested soybean leaves. Petitt and Smilowitz (1982) concluded that aphid feeding decreases the moisture content of infested leaves and plants under the stress of early-season infestation allocated more resources for leaf growth, but stem growth was severely retarded.

It is concluded that susceptible lines underwent more biotic stress through more lipid peroxidation through H_2O_2 production due to aphid infestation compared to tolerant lines of safflower. Tolerant safflower lines were had more ROS enzyme activity and total phenols than susceptible ones. Also, the tolerant lines are physiologically more efficient

with higher chlorophyll, net photosynthesis, net assimilation rate and intrinsic water use efficiency than the susceptible safflower lines.

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RESEARCH ARTICLE

Genetic Variability and Association Studies in Mid-late and Late Group of Cauliflower (*Brassica oleracea* L. var. *botrytis*)

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Abstract

Sufficient genetic variability was recorded in 26 genotypes based on various genetic variability parameters, indicating the scope for selection. High heritability and genetic advance for days to curd initiation, curd solidity, gross plant weight, net curd weight and marketable curd weight indicate the importance of additive gene action. Correlation and path analysis directed to focus selection on the basis of gross plant weight, harvest index, curd size index, curd solidity, curd depth and net curd weight to evolve high-yielding cauliflower genotypes. DPCaf US, DPCa CMS 1, DPCaf W3, DPCaf 30, DPCaf W3 and DPCaf US showed promise for curd yield, mainly attributed to the traits identified based on parameters of genetic variability.

Keywords: Cauliflower, Correlation coefficient, Heritability, Path coefficient.

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Introduction

Cauliflower, botanically known as *Brassica oleracea* L. var. *botrytis* with chromosome number $2n = 2x = 18$ and belongs to family Brassicaceae. Vegetable brassicas are rich in different bioactive compounds and phytochemicals. It is, therefore, important to identify promising genotypes with high productivity and increasing nutritional value (Ram *et al.*, 2017).

Knowledge about levels and patterns of genetic variability plays an important role in detecting diverse parents to generate segregating progenies with diverse backgrounds and introgression of desirable genes from variable germplasm into the existing genetic base. This implies that the extent of genetic variability in the germplasm is relatively proportional to the improvement potential of a crop and provides an opportunity to enhance the yield and quality through a planned breeding programme. Heritable characters, genetic parameters and association among traits are of prime importance in breeding (Sekhon and Sharma, 2019). The heritability estimates predict the consistency of phenotypic values in the expression of trait (Unche *et al.*, 2008).

Curd yield is a complex trait as many genes control it and hence are highly influenced by environmental conditions. It is crucial to understand the nature and magnitude of association between different yield components to improve the plant as a whole rather than the individual trait. The effective yield improvement would be achieved through the characters with significant and positive/desirable correlations. Some

of the component traits may be positively and directly associated with the marketable curd weight, thus can be used as a criterion of selection in the crop improvement programme. The association among the traits can be used for the selection of such traits whose genotypic values are modified by environmental effects and thus cannot be easily observed. It also gives information about the nature and extent of the direction and selection pressure among different characters (Sharma *et al.*, 2016). Genotypic and phenotypic correlations reveal the degree of association with different characters and helps to base the selection procedure when two opposite desirable characters affecting the principal character are to be selected. It also helps to improve different characters simultaneously. Thus, it would be imperative to examine the magnitude and direction of correlation of different attributes with marketable curd weight and to identify the traits of interest to obtain high yield. Correlation analysis indicates the association pattern of component traits with yield, thus showing inter-dependence. It simply represents the overall influence of a particular trait on yield rather than providing cause and effect relationship. The correlation *per se* does not provide the absolute picture of interrelationships when more than two variables are involved. The relative contribution of the component traits to the yield also provides the appropriate weightage for selection purpose. Therefore, path coefficient analysis is used to assess the cause-effect relationship and effective selection. It also helps in determining the degree of relationship of yield with its component effects and allows the critical examination of specific factors for a given correlation. It allows partitioning of correlation coefficients into direct and indirect effects of various traits towards dependent variable and plays an important role in determining the degree of relationship between yield and its component effects. A clear picture of each character's contribution toward the final expression of complex trait like marketable curd weight would emerge through the study of correlations coefficient and path coefficient analysis revealing different ways in which component attributes influence the complex characters.

Keeping this in mind, the present investigation was undertaken to develop a variety with high in marketable curd yield and quality through correlation and path analysis studies.

Materials and Methods

An experiment was conducted with 26 genotypes, including standard checks (Table 1) of cauliflower during 2018 and 2019 at the experimental farm of the Department of Vegetable science and floriculture, CSK HPKV, Palampur. The experimental material comprised of 26 genotypes of cauliflower, including standard checks (Table 1). The trial was laid out in randomized complete block design (RBD)

Table 1: List of genotypes

Genotype	Source
DPCa CMS 1, DPCa CMS 2, DPCa CMS 3, DPCaf 8, DPCaf 10, DPCaf 1, DPCaf 2, DPCaf W3, DPCaf US, DPCaf S5-1, DPCaY 1, DPCaY 7, DPCaf 9, DPCaf 12, DPCaf 13, DPCaf 18, DPCaf 24, DPCaf 29, DPCaf 30, DPCaf 35, Palam Uphar (Check)	Department of Vegetable Science & Floriculture, COA, CSKHPKV, Palampur
Pusa Paushja	ICAR-IARI, New Delhi
Pusa Himjyoti, Pusa Snowball KT-25, Pusa Snowball-1, Pusa Snowball K-1 (Check)	ICAR-IARI, Regional Station, Katrain, Kullu, HP

with three replications having 2.7 m row length at a spacing of 45 cm each within and between rows. The standard package of practices was followed to raise the healthy crop. The observations were recorded on randomly taken five competitive plants in each entry over replications during both the years for days to curd initiation, days to marketable curd maturity, stalk length (cm), leaves/plant, leaf length (cm), leaf width (cm), plant height (cm), plant frame (cm), curd depth (cm), curd diameter (cm), curd angle ($^{\circ}$), curd size index (cm^2), curd solidity (g/cm), gross plant weight (g), net curd weight (g), marketable curd weight (g), marketable curds (%), total soluble solids ($^{\circ}$ Brix) using hand refractometer and harvest index (%).

Statistical analysis of pooled data was subjected to analysis of genotypic coefficient variation (GCV), phenotypic coefficient of variation (PCV) and heritability (h^2) in broad sense were computed according to Burton and Devane, 1953. Genetic advance (GA) was estimated following Johnson *et al.*, 1955. The phenotypic and genotypic coefficients of correlation were worked out by the procedure of Al-Jibouri *et al.*, 1958; Dewey and Lu, 1959. The direct and indirect effects of component characters on marketable curd weight were computed using appropriate correlation coefficients of different component characters as suggested by Wright, 1921 and elaborated by Dewey and Lu, 1959.

Results and Discussion

The knowledge of phenotypic and genotypic coefficients of variation envisages the magnitude of variations present in the existing genetic material based on which the desirable breeding programme can be formulated for the genetic improvement of the target crop. Phenotypic coefficients of variation were greater than the genotypic coefficient variation values for all the characters studied (Table 2). Therefore, caution has to be exercised in making selection for these characters on the basis of phenotype alone since environment had also its role in influencing the trait of interest. Moderate PCV and GCV was recorded for days to curd initiation (17.42, 17.69), days to marketable curd maturity (13.41, 14.84), plant frame (11.52, 13.55) and curd size index (11.55, 13.46), curd solidity (24.4, 55.99), gross plant weight

Table 2: Genetic parameters of variability for different agro-morphological traits of cauliflower

Traits	GCV (%)	PCV (%)	GCV (%)	PCV (%)	GCV (%)	PCV (%)	h^2_{bs}			GA (%)		
	2018-19		2019-20		pooled		2018-19	2019-20	pooled	2018-19	2019-20	pooled
Days to curd initiation	13.56	13.89	11.78	12.20	17.42	17.69	95.42	93.16	96.94	27.29	23.42	34.98
Days to marketable curd maturity	10.13	12.96	8.75	9.77	13.41	14.84	61.09	80.21	81.83	16.31	16.14	24.76
Stalk length (cm)	5.43	8.45	10.87	13.89	7.92	10.85	41.31	61.24	53.33	7.19	17.52	11.76
Leaves/plant	8.66	11.28	5.62	8.04	9.14	11.22	59.05	48.82	66.46	13.72	8.09	15.20
Leaf length (cm)	5.69	8.85	7.40	9.92	8.52	10.84	41.33	55.58	61.86	7.54	11.36	13.63
Leaf width (cm)	6.61	10.80	7.28	11.22	9.27	12.62	37.46	42.06	53.97	8.34	9.73	13.80
Plant height (cm)	6.72	9.21	8.20	11.91	9.95	12.56	53.29	47.48	62.80	10.11	11.65	16.03
Plant frame (cm)	7.54	10.53	10.49	12.57	11.52	13.55	51.29	69.61	72.30	11.13	18.02	20.09
Curd depth (cm)	4.80	6.58	4.92	6.08	6.30	7.45	53.24	65.42	71.42	7.21	8.19	10.80
Curd diameter (cm)	4.07	5.28	5.81	7.21	5.93	7.07	59.51	65.01	70.37	6.47	9.65	10.14
Curd angle ($^{\circ}$)	2.70	4.78	3.28	4.05	3.71	4.92	31.75	65.55	57.00	3.13	5.47	5.79
Curd size index (cm ²)	7.46	10.35	10.02	12.04	11.55	13.46	51.96	69.27	73.61	11.08	17.18	20.23
Curd solidity (g/cm)	23.06	24.57	15.20	16.42	24.94	25.99	88.10	85.75	92.08	44.59	29.00	49.25
Gross plant weight (g)	16.24	16.71	16.00	17.10	21.76	22.37	94.50	87.55	94.60	32.52	30.84	43.31
Net curd weight (g)	25.01	26.52	17.59	18.62	28.26	29.21	88.93	89.24	93.58	48.58	34.22	55.99
Marketable curd weight (g)	18.12	18.81	16.52	17.86	22.81	23.61	92.78	85.60	93.36	35.96	31.49	45.23
Marketable curds (%)	4.92	10.25	6.18	9.92	9.17	12.42	23.03	38.77	54.43	4.86	7.93	13.82
Total soluble solids ($^{\circ}$ Brix)	5.59	6.46	5.32	6.74	7.12	8.03	74.92	62.29	78.57	9.97	8.65	12.88
Harvest index (%)	5.02	6.44	4.39	6.65	5.81	7.38	60.83	43.59	62.03	8.07	5.97	9.42

(21.76, 22.37), net curd weight (28.26, 29.21) and marketable curd weight (22.81, 23.61) on pooled basis, respectively suggesting their improvement by exploiting heterosis or following hybridization programme. Earlier workers have also found moderate PCV and GCV for stalk length and curd

size index (Dubey *et al.*, 2003), net curd weight (Kumar *et al.*, 2011), marketable curd weight and gross plant weight (Chittora and Singh, 2015).

Heritability is a component which computes expected progress but it becomes more meaningful when

Table 3: Estimates of phenotypic (P) and genotypic (G) correlation coefficients for different traits in cauliflower (pooled over years)

Traits	Days to initiation	Days to curd	Days to marketable curd maturity	Stalk Length (cm)	Leaves/plant	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	Plant Frame (cm)	Curd depth (cm)	Curd diameter (cm)	Curd Angle (°)	Marketable curds (%)	Total soluble solids (°Brix)	Harvest index (%)	Curd solidity (g/cm)	Curd Size index (cm ²)	Gross plant weight (g)	Net curd Weight (g)
Days to marketable curd maturity	P 0.844*																		
G 0.928*																			
Stalk length (cm)	P -0.315*		-0.368*																
G -0.436*			-0.408*																
Leaves/plant	P -0.357*		-0.341*	-0.013															
G -0.463*			-0.470*	0.017															
Leaf length (cm)	P 0.161		0.054	0.147	-0.012														
G 0.225*			0.252*	0.090	-0.243*														
Leaf width (cm)	P 0.089		0.090	0.201	-0.247*	0.501*													
G 0.102			0.217	0.289*	-0.667*	0.436*													
Plant height (cm)	P 0.191		0.134	0.181	-0.261*	0.750*	0.440*												
G 0.259*			0.247*	0.201	-0.506*	0.852*	0.476*												
Plant frame (cm)	P -0.261*		-0.345*	0.291*	-0.165	0.490*	0.499*	0.562*											
G -0.328*			-0.407*	0.448*	-0.383*	0.585*	0.585*	0.654*											
Curd depth (cm)	P 0.370*		0.199	-0.077	0.251*	0.351*	0.096	0.309*	0.053										
G 0.449*			0.318*	-0.114	0.166	0.397*	-0.028	0.346*	-0.042										
Curd diameter (cm)	P 0.064		-0.113	-0.072	0.196	0.329*	0.131	0.340*	0.293*	0.655*									
G 0.093			-0.072	-0.173	0.151	0.285*	-0.100	0.306*	0.185	0.716*									
Curd angle (°)	P 0.458*		0.316*	-0.069	-0.019	0.082	0.061	0.029	-0.103	0.518*	0.375*								
G 0.626*			0.588*	-0.024	-0.108	-0.082	-0.013	0.047	-0.200	0.547*	0.543*								
Marketable curds (%)	P -0.484*		-0.471*	0.054	0.247*	-0.007	-0.025	-0.116	0.119	0.058	0.389*	0.017							
G -0.652*			-0.697*	-0.033	0.546*	-0.158	-0.172	-0.352*	0.300*	0.031	0.553*	-0.019							
Total soluble solids (°Brix)	P -0.377*		-0.412*	0.150	-0.048	-0.126	0.146	-0.057	0.327*	-0.039	0.234*	-0.147	0.389*						
G -0.442*			-0.522*	0.196	-0.108	-0.211	0.219	-0.136	0.443*	-0.013	0.267*	-0.185	0.586*						
Harvest index (%)	P -0.034		-0.143	0.089	0.170	-0.223*	-0.265*	-0.200	-0.019	0.233*	0.258*	0.260*	0.329*	0.036					
G -0.019			-0.199	-0.023	0.270*	-0.402*	-0.418*	-0.301*	-0.015	0.299*	0.283*	0.405*	0.367*	-0.005					
Curd solidity (g/cm)	P 0.199		0.208	0.047	-0.054	0.036	0.041	0.090	0.163	0.301*	0.418*	0.584*	0.173	0.067	0.306*				
G 0.222			0.244*	0.054	-0.078	0.064	0.072	0.137	0.203	0.421*	0.539*	0.852*	0.261*	0.109	0.412*				
Curd size index (cm ²)	P 0.241*		0.050	-0.081	0.241*	0.367*	0.130	0.355*	0.191	0.913*	0.904*	0.496*	0.241*	0.110	0.268*	0.397*			
G 0.295*			0.133	-0.147	0.164	0.365*	-0.052	0.353*	0.081	0.930*	0.921*	0.595*	0.314*	0.144	0.312*	0.517*			
Gross plant weight (g)	P 0.205		0.238*	0.060	0.116	0.324*	0.263*	0.313*	0.214	0.604*	0.541*	0.570*	0.137	0.105	0.000	0.724*	0.637*		
G 0.219			0.286*	0.140	0.087	0.351*	0.261*	0.325*	0.181	0.710*	0.601*	0.759*	0.198	0.122	0.059	0.754*	0.715*		
Net curd weight (g)	P 0.274*		0.232*	0.020	0.014	0.122	0.063	0.168	0.164	0.534*	0.555*	0.653*	0.174	0.061	0.334*	0.966*	0.602*	0.804*	
G 0.299*			0.280*	0.017	-0.035	0.146	0.062	0.207	0.174	0.607*	0.650*	0.883*	0.247*	0.107	0.430*	0.976*	0.679*	0.830*	
Marketable curd weight (g)	P 0.186		0.181	0.075	0.163	0.220	0.157	0.221	0.194	0.642*	0.590*	0.623*	0.233*	0.121	0.320*	0.796*	0.683*	0.946*	0.878*
G 0.207			0.224*	0.113	0.143	0.205	0.124	0.214	0.163	0.741*	0.638*	0.827*	0.284*	0.126	0.320*	0.837*	0.750*	0.964*	0.909*

*Significant at $P \leq 0.05$

accompanied by genetic advance. Prediction on the basis of heritability and genetic advance estimates could be more useful in selection (Sharma *et al.*, 2016). High heritability along with high genetic advance was reported for days to curd initiation (96.94, 34.98), curd solidity (92.08, 49.25), gross plant weight (94.60, 43.31), net curd weight (93.58, 55.99) and marketable curd weight (93.36, 45.23) in pooled over years, respectively that indicated the importance of additive gene action for the inheritance of these traits and improvement can be possible through phenotypic selection for these traits in the early generation (Sharma *et al.*, 2006; Kumar *et al.*, 2018).

The correlation coefficient estimates degree of association between two traits worked out at the same time. The correlation studies between various curd yield attributes and curd yield provide a basis for further breeding programmes in cauliflower. The results of the experiment revealed that the genotypic and phenotypic correlation coefficient was similar in directions, while in magnitude, genotypic correlations were mostly higher than corresponding phenotypic correlations. The low phenotypic correlation could result from the masking and modifying effect of environment factors on the association of traits at the genotypic level. At phenotypic and genotypic levels, the marketable curd weight had a positive and significant correlation with curd depth (0.642, 0.740), curd diameter (0.590, 0.638), curd angle (0.623, 0.827), marketable curds (%) (0.233, 0.284), harvest index (0.320, 0.32), curd solidity (0.796, 0.837), curd size index (0.683, 0.750), gross plant weight (0.946, 0.964) and net curd weight (0.878, 0.909), respectively on pooling data over the years. Besides, days to marketable curd maturity shows a positive and significant correlation with marketable curd weight at the genotypic level only (Table 3 and Figure 1). Earlier researchers have also reported significant and positive association of marketable curd weight with different curd attributes in their respective studies in different environments (Sharma *et al.*, 2006; Singh *et al.*, 2014; Shruthy and Celine, 2016; Vanlalneihi *et al.*, 2017).

The intercorrelation between marketable curd weight contributing characters may affect the selection for component characters either in favourable or unfavorable direction. A significant and positive association of leaf length, leaf width, plant height and plant frame were recorded among themselves at both phenotypic and genotypic levels. Further, critical insight into the correlation coefficients showed that curd depth, curd diameter, curd angle, harvest index, curd solidity, curd size index, gross plant weight and net curd weight showed a significant and positive association with one another at both phenotypic and genotypic.

On the basis of correlation studies and their coefficients of determination, it can be concluded that leaf length, leaf width, plant height, curd depth, curd diameter, curd

angle, harvest index, curd solidity, curd size index, gross plant weight and net curd weight should be taken into consideration while isolating plants with high marketable curd weight. In addition, days to curd initiation and marketable curd maturity would be given due consideration for selecting early/late maturing genotypes within the maturity group. The majority of these traits showed a correlation in the same direction over the years, emphasizing their importance for the improvement of cauliflower.

In order to understand the cause of the correlations among the traits studied, the estimates of a direct and indirect contribution of different traits towards marketable curd weight, the path coefficient analysis was done (Table 4). The direct effects at a genotypic level were different than that at a phenotypic level. These differences might be due to varying degree of influence of environment on various traits studied, which were also observed from the results of component variance analysis and correlation studies. In few cases, the direct effects were observed to be of opposite sign (positive to negative and vice-versa) at corresponding phenotypic and genotypic levels like in days to curd initiation, leaves/plant, leaf length, leaf width, plant height, plant frame, curd depth, curd diameter, curd angle, curd size index, curd solidity, net curd weight and total soluble solids. Such a change in direction and magnitude of direct and indirect effects might be due to environmental factors influencing various traits. Therefore, path analysis at the phenotypic level may not provide a true picture of direct and indirect causes. It would be advisable to understand the contribution of different traits towards the marketable curd weight at the genotypic level. Gross plant weight had maximum positive and direct effect on marketable curd weight (0.923/ 1.042) followed by harvest index (0.308/0.152) at phenotypic and genotypic levels, respectively. Net curd weight also contributed significantly to the total association directly at a phenotypic level in pooled years. Besides, leaf length and curd solidity in pooled years also showed a good magnitude of direct effects at genotypic level only. Earlier workers have also reported positive and direct effects of different traits with marketable curd weight (Liu *et al.*, 2004; Sharma *et al.*, 2006; Sheemar *et al.*, 2012; Vanlalneihi *et al.*, 2017).

The significant and positive association of marketable curd weight with days to marketable curd maturity, curd depth, curd diameter, curd angle, curd size index, curd solidity, gross plant weight, net curd weight, marketable curds (%) and harvest index was due to indirect effect via gross plant weight followed by harvest index both at phenotypic and genotypic levels, curd size index at genotypic level only in pooled over years along with curd solidity at genotypic level. On the contrary, net curd weight reduced the magnitude of total association of marketable curd weight with most traits due to its negative indirect

Table 4: Estimates of direct and indirect effects of different traits on marketable curd weight at phenotypic (P) and genotypic (G) levels in cauliflower (pooled over years)

Traits	Days to Curd initiation	Days to Marketable curd maturity	Stalk length (cm)	Leaves/ plant	Leaf length (cm)	Leaf width (cm)	Plant Height (cm)	Plant Frame (cm)	Curd depth (cm)	Curd diameter (cm)	Curd angle (°)	Curd size index (cm ²)	Curd solidity (g/cm)	Gross plant weight (g)	Net curd weight (g)	Marketable curds (%)	Total soluble solids (°Brix)	Harvest index (%)	r
Days to curd initiation	P 0.012	-0.012	0.004	-0.003	-0.002	0.000	0.000	0.000	0.018	0.005	-0.003	-0.046	-0.041	0.190	0.077	0.002	-0.005	-0.011	0.186
	G -0.371	-0.140	0.055	0.276	0.067	-0.051	-0.073	0.014	-2.375	-0.634	0.012	3.777	1.299	0.228	-1.966	0.037	0.055	-0.003	0.207
Days to marketable curd maturity	P 0.011	-0.014	0.004	-0.003	-0.001	0.000	0.000	0.000	0.010	-0.009	-0.002	-0.009	-0.043	0.220	0.065	0.002	-0.006	-0.044	0.181
	G -0.344	-0.151	0.051	0.280	0.075	-0.108	-0.070	0.018	-1.68	0.491	0.011	1.699	1.427	0.297	-1.846	0.039	0.065	-0.030	0.224*
Stalk length (cm)	P -0.004	0.005	-0.012	0.000	-0.001	0.001	0.00	0.000	-0.004	-0.006	0.000	0.015	-0.010	0.055	0.006	0.000	0.002	0.027	0.075
	G 0.162	0.062	-0.125	-0.010	0.027	-0.144	-0.057	-0.019	0.604	1.175	0.000	-1.882	0.317	0.145	-1.115	0.002	-0.024	-0.004	0.113
Leaves/plant	P -0.004	0.005	0.000	0.009	0.000	-0.001	0.000	0.000	0.012	0.016	0.000	-0.046	0.011	0.107	0.004	-0.001	-0.001	0.052	0.163
	G 0.172	0.071	-0.002	-0.596	-0.072	0.331	0.142	0.016	-0.876	-1.028	-0.002	2.098	-0.457	0.091	0.231	-0.031	0.013	0.041	0.143
Leaf length (cm)	P 0.002	-0.001	-0.002	0.000	-0.01	0.002	0.001	0.000	0.017	0.027	-0.001	-0.070	-0.007	0.299	0.034	0.000	-0.002	-0.069	0.22
	G -0.083	-0.038	-0.011	0.145	0.297	-0.217	-0.240	-0.025	-2.100	-1.933	-0.002	4.660	0.375	0.366	-0.963	0.009	0.026	-0.061	0.205
Leaf width (cm)	P 0.001	-0.001	-0.002	-0.002	-0.005	0.004	0.000	0.000	0.005	0.011	0.000	-0.025	-0.008	0.243	0.018	0.000	0.002	-0.082	0.157
	G -0.038	-0.033	-0.036	0.398	0.129	-0.497	-0.134	-0.025	0.145	0.679	0.000	-0.668	0.423	0.272	-0.410	0.01	-0.027	-0.064	0.124
Plant height (cm)	P 0.002	-0.002	-0.002	-0.002	-0.008	0.002	0.001	0.000	0.015	0.027	0.000	-0.068	-0.019	0.289	0.047	0.000	-0.001	-0.062	0.221
	G -0.096	-0.037	-0.025	0.302	0.253	-0.236	-0.282	-0.028	-1.829	-2.079	0.001	4.507	0.802	0.338	-1.366	0.020	0.017	-0.046	0.214
Plant frame (cm)	P -0.003	0.005	-0.004	-0.001	-0.005	0.002	0.001	0.001	0.003	0.024	0.001	-0.037	-0.033	0.198	0.046	0.000	0.005	-0.006	0.194
	G 0.122	0.062	-0.056	0.228	0.174	-0.291	-0.184	-0.043	0.224	-1.258	-0.004	1.036	1.185	0.189	-1.145	-0.017	-0.055	-0.002	0.163
Curd depth (cm)	P 0.005	-0.003	0.001	0.002	-0.004	0.000	0.000	0.000	0.048	0.053	-0.003	-0.175	-0.062	0.558	0.150	0.000	-0.001	0.072	0.642*
	G -0.167	-0.048	0.014	-0.099	0.118	0.014	-0.097	0.002	-5.287	-4.86	0.010	11.889	2.464	0.740	-3.997	-0.002	0.002	0.045	0.741*
Curd diameter (cm)	P 0.001	0.002	0.001	0.002	-0.003	0.000	0.000	0.000	0.031	0.081	-0.002	-0.173	-0.086	0.499	0.156	-0.001	0.003	0.080	0.590*
	G -0.035	0.011	0.022	-0.090	0.085	0.050	-0.086	-0.008	-3.785	-6.789	0.010	11.778	3.150	0.627	-4.28	-0.031	-0.033	0.043	0.638*
Curd angle (°)	P 0.006	-0.004	0.001	0.000	-0.001	0.000	0.000	0.000	0.025	0.030	-0.006	-0.095	-0.120	0.526	0.184	0.000	-0.002	0.080	0.623*
	G -0.232	-0.089	0.003	0.064	-0.024	0.007	-0.013	0.009	-2.892	-3.683	0.019	7.610	4.983	0.791	-5.811	0.001	0.023	0.062	0.827*
Curd size index (cm ²)	P 0.003	-0.001	0.001	0.002	-0.004	0.000	0.000	0.000	0.044	0.073	-0.003	-0.191	-0.082	0.587	0.170	-0.001	0.002	0.082	0.683*
	G -0.110	-0.020	0.018	-0.098	0.108	0.026	-0.099	-0.003	-4.917	-6.256	0.011	12.782	3.022	0.744	-4.470	-0.018	-0.018	0.047	0.750*
Curd solidity (g/cm)	P 0.002	-0.003	-0.001	0.000	0.000	0.000	0.000	0.000	0.014	0.034	-0.004	-0.076	-0.206	0.668	0.272	-0.001	0.001	0.094	0.796*
	G -0.082	-0.037	-0.007	0.047	0.019	-0.036	-0.039	-0.009	-2.228	-3.658	0.016	6.608	5.846	0.786	-6.423	-0.015	-0.014	0.063	0.837*
Gross plant weight (g)	P 0.003	-0.003	-0.001	0.001	-0.003	0.001	0.000	0.000	0.029	0.044	-0.004	-0.122	-0.149	0.923	0.226	-0.001	0.001	0.000	0.946*
	G -0.081	-0.043	-0.017	-0.052	0.104	-0.130	-0.091	-0.008	-3.755	-4.083	0.014	9.133	4.410	1.042	-5.461	-0.011	-0.015	0.009	0.964*
Net curd weight (g)	P 0.003	-0.003	0.000	0.000	-0.001	0.000	0.000	0.000	0.026	0.045	-0.004	-0.115	-0.199	0.742	0.282	-0.001	0.001	0.103	0.878*
	G -0.111	-0.042	-0.002	0.021	0.043	-0.031	-0.058	-0.007	-3.210	-4.414	0.017	8.680	5.704	0.864	-6.583	-0.014	-0.013	0.065	0.909*
Marketable curds (%)	P -0.006	0.006	-0.001	0.002	0.000	0.000	0.000	0.000	0.003	0.031	0.000	-0.046	-0.035	0.127	0.049	-0.004	0.005	0.101	0.233*
	G 0.242	0.105	0.004	-0.326	-0.047	0.085	0.099	-0.013	-0.165	-3.752	0.000	4.017	1.525	0.206	-1.624	-0.056	-0.073	0.056	0.284*
Total soluble solids (°Brix)	P -0.005	0.006	-0.002	0.000	0.001	0.001	0.000	0.000	-0.002	0.019	0.001	-0.021	-0.014	0.096	0.017	-0.001	0.014	0.011	0.121
	G 0.164	0.079	-0.025	0.064	-0.063	-0.109	0.038	-0.019	0.069	-1.810	-0.004	1.841	0.636	0.127	-0.705	-0.033	-0.125	-0.001	0.126
Harvest index (%)	P 0.000	0.002	-0.001	0.001	0.002	-0.001	0.000	0.000	0.011	0.021	-0.002	-0.051	-0.063	0.000	0.094	-0.001	0.001	0.308	0.320*
	G 0.007	0.030	0.003	-0.161	-0.119	0.208	0.085	0.001	-1.581	-1.923	0.008	3.990	2.409	0.062	-2.830	-0.021	0.001	0.152	0.320*

Residual effect at phenotypic level (p) = 0.00168 and genotypic level (G) = -0.00767
 *Significant at P ≤ 0.05; bold values indicate direct effects; r: correlation coefficient with marketable curd weight

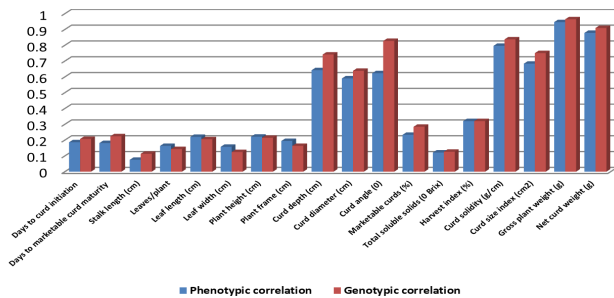


Figure 1: Genotypic and phenotypic correlation of marketable curd weight with 18 characters in cauliflower (pooled over years)

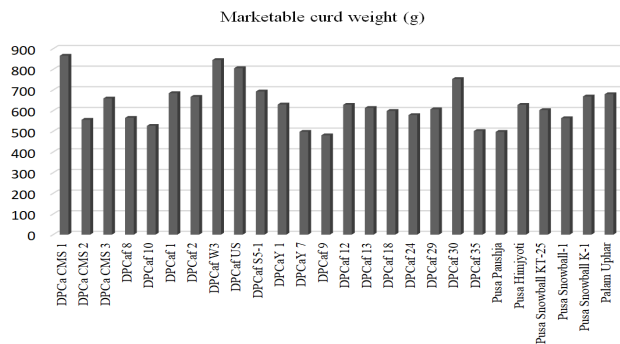


Figure 2: Performance of genotypes for marketable curd weight (pooled over years)

contribution. The magnitude of unexplained variation for marketable curd weight was very low at phenotypic (0.00168) and genotypic (-0.00767) levels suggesting that the traits included in the present investigation accounted for the substantial part of the variation present in the dependable variation i.e. marketable curd weight. In view of the direct and indirect contribution of component traits, selection on the basis of gross plant weight along with harvest index, curd size index, curd solidity, curd depth and net curd weight would be a rewarding proposition for evolving high-yielding cauliflower genotypes.

Genotypes namely, DPCaf US, DPCa CMS 1, DPCaf W3 and DPCaf 30 showed good marketable curd yield (Figure 2) that was mainly the result of better curd depth, curd diameter, leaf length, leaf width, leaves/plant, optimum plant frame, curd compactness, curd solidity, gross plant weight and net curd weight. Besides, DPCaf W3 and DPCaf US had better harvest index than DPCa CMS 1 and DPCaf 30 which may be attributed to moderate plant frame, leaf length, leaf width, and height along with short stalk length. Therefore, it is quite apparent from the results that inference drawn on the basis of heritability, genetic advance and correlation studies that focus on these traits have direct bearing on the marketable curd yield and to create genetic variability in the existing germplasm pool.

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SHORT COMMUNICATION

Flow Cytometric Based Estimation of Genome Size in Arecanut (*Areca catechu* L.) Varieties

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Abstract

The present work was undertaken with the objective to estimate the nuclear DNA content in arecanut varieties using flow cytometry. Released varieties of arecanut maintained in the field gene bank at ICAR-Central Plantation Crops Research Institute (ICAR-CPCRI), Regional Station, Vittal, Karnataka, were used for the estimation of DNA content by using BD AccuriC6 flow cytometer. Nuclei isolated from leaves of *Pisum sativum* cv. Citrad of known genome size was used as an external reference standard. Significant differences were observed between the arecanut varieties for 2C DNA content. Among the arecanut varieties, 2C DNA content (genome size) was found to be ranging from 6.025 (2.946 Gb/1C) to 6.710 pg (3.281 Gb/1C), with mean value of 6.472 pg (3.164 Gb/1C). The highest DNA content was recorded in the variety Swarnamangala (6.710 pg/2C or 3.281 Gb/1C), followed by Sumangala (6.695 pg/2C or 3.273 Gb/1C) and Shatamangala (6.623 pg/2C or 3.238 Gb/1C), while the lowest DNA content of 6.025 pg/2C or 2.946 Gb/1C was recorded in the variety Sreemangala. The results of this study indicate the potential of using flow cytometry for studying genome size diversity in arecanut.

Keywords: Flow cytometry, *Areca catechu*, Varieties, DNA content, Genome size.

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Introduction

The areca palm which is highly cross-pollinated is an allotetraploid with chromosome number $2n = 32$ (Venkatasubban, 1945). The palm is an unbranched, erect, monoecious tree growing in hot and humid tropical region of the world and its centre of origin is considered to be South East Asia (Bavappa *et al.*, 1982). It is the lone commercially cultivated crop majorly used for masticatory purposes and also in socio-religious purposes since thousands of years (Balasimha and Rajagopal, 2004). The arecanut chewed along with betel leaf and slaked lime for their effect as a mild stimulant (Rao, 1982), increases stamina, and alertness, kills worms, removes/subdues bad odor, beautifies the mouth, induces purification and causes mild sensation in the body and stimulates flow of saliva to aid digestion. The nuts contain 8 to 12% fat which can be mixed with cocoa fat for confectionery products and also substituted for vanaspati in preparations of sweets and biscuits (Murthy, 1957).

Extensive research works in arecanut was carried out on morphological and biochemical characterization in order to make use of these characters in crop improvement programmes. However, the genetic diversity information provided by morphological characters is limited and environmental and physiological factors can influence these parameters. Therefore, researchers are using molecular markers. In arecanut, molecular markers like random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs) etc. have been used to study the genetic relationships, diversity and geographical correlation among different arecanut germplasm,

varieties, wild species and related genera. However, no work on genome size diversity has been undertaken in arecanut.

Flow cytometry studies have been undertaken in different crop species in different parts of the world. There are very few reports available on such studies in the family Arecaceae, while reports of such works are not available in arecanut as per our knowledge. Therefore, the present work was undertaken with the objective to estimate the DNA content or genome size in arecanut varieties using flow cytometry.

To estimate 2C DNA content, nine released arecanut varieties (five varieties developed by evaluation of indigenous germplasm and four varieties developed by evaluating exotic accessions), maintained in the field gene bank at ICAR-Central Plantation Crops Research Institute, Regional Station, Vittal, Karnataka (ICAR-CPCRI) (Table 1) were used. Spindle leaves collected from three palms in each variety were used to isolate the intact nuclei. The leaves from *Pisum sativum* cv. Citrad of known genome size ($2n = 9.09$ pg) was used as a reference standard (Dolezel and Bartos, 2005).

Estimation of nuclear DNA content was done by using BD Accuri C6 flow cytometer available at Division Crop Improvement, ICAR-CPCRI, Kasaragod, Kerala. Galbraith *et al.* (1983) explained procedure used for the flow cytometry analysis. To isolate intact nuclei, 100 mg of leaf tissue of individual palms from each arecanut variety and 50 mg of pea leaves (external reference standard) were chopped separately with a sharp razor blade for 90 seconds in a petri dish containing 1.5 mL of cold nuclear isolation buffer supplemented with β -mercaptoethanol and 1% PVP (in order to reduce the effect of phenolic compounds). After chopping, the buffer containing cell constituents and large tissue remnants was passed sequentially through nylon filters with mesh size of 50 μ m followed by 20 μ m to separate nuclei from the cell debris. To the buffer with nuclei, 2.5 μ L of 10 mg/mL of DNase free RNase A and flurochrome propidium iodide were added to a final concentration of 50 μ g/mL. Then the samples were incubated at 4°C under dark condition for 15 minutes before flow cytometric analysis. During the preparation of reference standard (pea) and arecanut samples same experimental conditions are maintained to avoid the error since pea is used as an external reference standard. External standardization was followed since internal standardization yielded almost overlapping histogram peaks and higher coefficient of variation (CV).

Before estimating the nuclear DNA content in arecanut varieties, two nuclei isolation buffers such as Galbraith's buffer (Galbraith *et al.*, 1983) and LB01 buffer (Dolezel *et al.*, 1989) were screened in order to isolate quality nuclear suspension for flow cytometry analysis.

The samples were analyzed on a BD AccuriC6 flow cytometer with an excitation wavelength of 488 nm. In each variety, three samples with three replications were

analyzed with low speed and counted up to 1500 nuclei per sample using C6 flow plus software. Gunn *et al.* (2015) and Ranjini *et al.* (2020) counted up to 500 and 1500 nuclei per sample, respectively. Based on these references in the present experiment up to 1500 nuclei are counted for each sample. Reference standard (pea) was analyzed each time before analyzing every arecanut sample. Further, mean fluorescent units for both reference standard (pea) and arecanut were recorded based on the position of the G0/G1 peak (non-replicated phase of the cell cycle) and 2C nuclear DNA content was calculated as per the Dolezel *et al.* (2003) and expressed in pg/2C.

$$2C \text{ value of the arecanut sample} = \frac{G0/G1 \text{ peak value of the arecanut}}{G0/G1 \text{ peak value of the reference standard}} \times \text{Genome size of the reference standard (2C=9.09 pg)}$$

The 2C DNA content was converted to mega base-pairs (Mbp) by a conversion factor of 1 pg = 978 Mbp (Dolezel *et al.*, 2003). The values were statistically tested and a post hoc test like Duncan's multiple range test (DMRT) was done by employing IBM SPSS Statistics version 20 Software.

In tropical perennial tree species like arecanut, such studies are rare, hence this study was carried out to determine genome size in this palm using a flow cytometric procedure in arecanut varieties, comprising of five varieties developed and released by evaluating indigenous germplasm and four varieties developed and released by evaluating exotic accessions, maintained in the field gene bank at ICAR-CPCRI, Regional Station, Vittal, Karnataka.

Among the two nuclei isolation buffers tested for obtaining quality nuclear suspension viz., Galbraith's buffer (Galbraith *et al.*, 1983) and LB01 buffer (Dolezel *et al.*, 1989), arecanut samples prepared using LB01 buffer gave better results in terms of sufficient nuclei yield, lesser background noise and lowest coefficient of variation of histogram of G0/G1 peak (2.5%) as compared to the samples prepared in the Galbraith buffer. Galbraith *et al.*, (1983) opined that in most cases histograms with peak CVs below 3% is considered fully acceptable. Hence for the estimation of 2C DNA content in arecanut varieties, intact nuclei isolated from the LB01 buffer was used (Figure 1). CVs of histogram of G0/G1 peak in all the arecanut varieties were maintained below 2.5%.

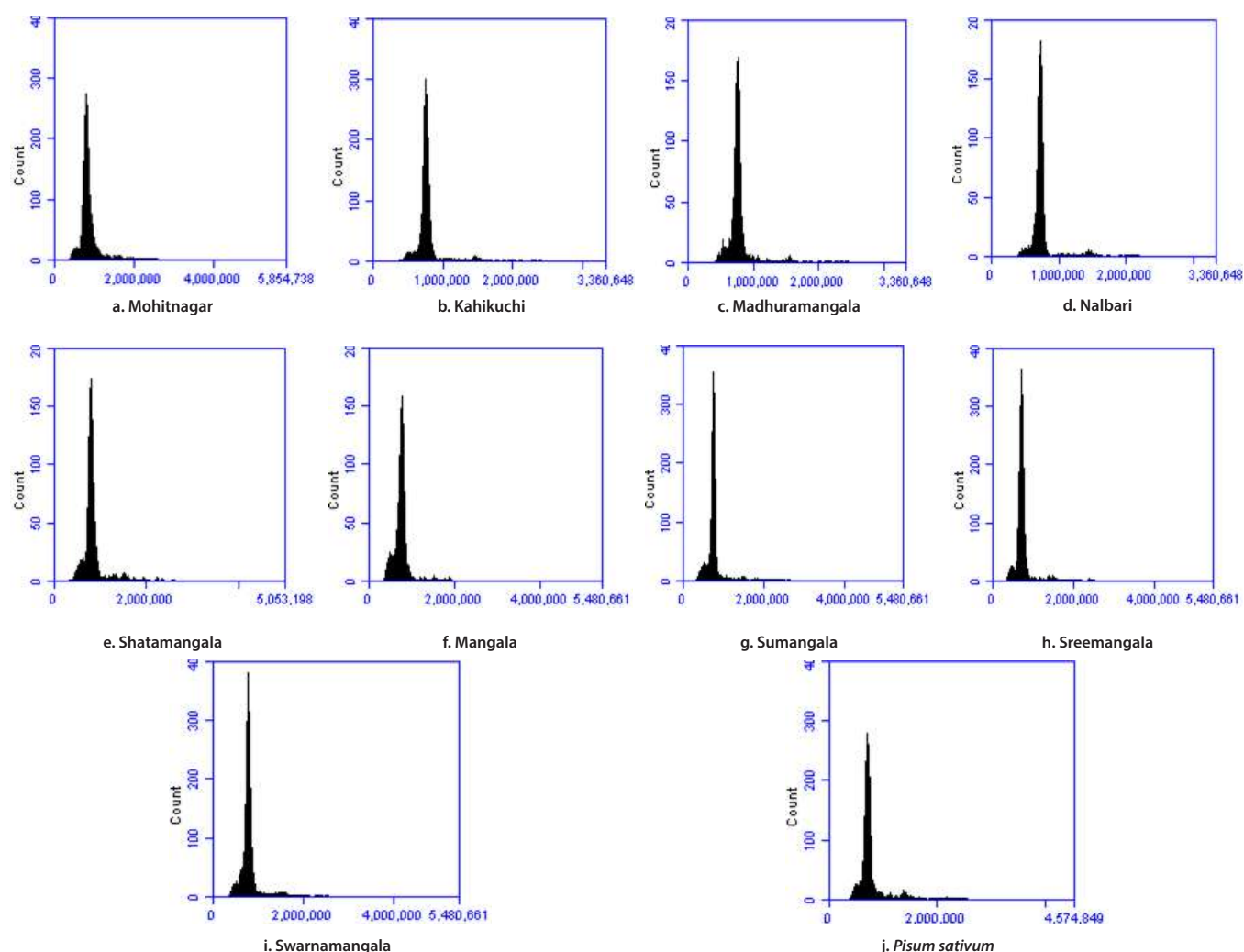
Among all the nine arecanut varieties studied in the present investigation, the 2C DNA content (genome size) varied from 6.025 pg (2.946 Gb/1C) to 6.710 pg (3.281 Gb/1C). Mean DNA content was observed to be 6.472 pg/2C (3.164 Gb/1C) (Table 1). The analysis of variance (ANOVA) indicated significant differences among arecanut varieties for nuclear DNA content (Table 2). The highest DNA content (genome size) was observed in the variety Swarnamangala, 6.710 pg/2C (3.281 Gb/1C), followed by Sumangala 6.695 pg/2C (3.273 Gb/1C), Shatamangala (6.623 pg/2C or 3.238 Gb/1C) and the lowest DNA content (genome size) of 6.025 pg/2C (2.946 Gb/1C) was recorded from the variety Sreemangala.

Table 1: Arecanut varieties used in the study and DNA content of arecanut varieties estimated by flow cytometry

Varieties	Source of germplasm	DNA content (pg/2C)	Genome size (Mbp/haploid set)
Mohitnagar	India (IC 557422)	6.557 $\pm$ 0.019 ^{bc}	3206.536
Kahikuchi	India (VTL-64)	6.340 $\pm$ 0.045 ^e	3100.423
Madhuramangala	India (IC 593737)	6.491 $\pm$ 0.007 ^{cd}	3173.936
Nalbari	India (IC 593736)	6.363 $\pm$ 0.015 ^e	3111.670
Shatamangala	India (IC 557397)	6.623 $\pm$ 0.036 ^b	3238.810
Mangala	China (IC 557417)	6.439 $\pm$ 0.006 ^d	3148.671
Sumangala	Indonesia (IC 557418)	6.695 $\pm$ 0.043 ^a	3273.692
Sreemangala	Singapore (IC 557420)	6.025 $\pm$ 0.072 ^f	2946.225
Swarnamangala	Vietnam (IC 557419)	6.710 $\pm$ 0.002 ^a	3281.190
Mean		6.472 $\pm$ 0.215	3164.573

Table 2: ANOVA of DNA content (pg/2C) in arecanut varieties

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Significance level (p-value: 0.05)
Between Groups	8	1.100	0.138	89.849	0.000
Within Groups	18	0.028	0.002		
Total	26	1.128			

**Figure 1:** Flow cytometry histogram of arecanut varieties and reference standard.

DMRT post hoc test also indicated significant differences among arecanut varieties for 2C DNA content. The DNA content of the Sreemangala variety is significantly different from that of all other remaining varieties. DNA content of Swarnamangala and Sumangala is found to be significantly different from all other arecanut varieties. Similarly, 2C values of Shatamangala and Mohitnagar, Mohitnagar and Madhuramangala, Madhuramangala and Mangala and Nalbari and Kahikuchi were significantly different from remaining varieties (Table 1).

The intraspecific variation observed for nuclear DNA content in arecanut corresponds to intraspecific variation reported for other palm species. Genome size of 23 coconut populations covering most of the genetic diversity of the genus *Cocos nucifera* was analyzed by flow cytometer and reported the presence of intraspecific variation for nuclear DNA content in coconut with mean nuclear DNA content of 5.966 ± 0.111 pg/2C (Gunn *et al.*, 2015). Further, similar observations were made in coconut by Neto *et al.* (2016). In oil palm, Madon *et al.* (2008) estimated the genome size of *Elaeis sp.* and its intra-specific and inter-specific hybrids using *Glycine max* as external standard. The average 2C DNA content was found to be 3.86 ± 0.26 pg. They found that there was a great variation between intra and inter-specific hybrids of *Elaeis* in their nuclear DNA content. Similarly, Camillo *et al.* (2014) observed that African oil palm has a nuclear DNA content of 4.32 ± 0.173 pg and American oil palm a nuclear DNA content of 4.43 ± 0.018 pg, and concluded that the genome size of American oil palm is larger than that of African oil palm.

Among the varieties of arecanut developed from indigenous germplasm, 2C DNA content (genome size) ranged from 6.340 pg/2C (3.100 Gb/1C) to 6.623 pg/2C (3.238 Gb/1C) with a mean DNA content of 6.475 pg/2C (3.166 Gb/1C). The highest DNA content (genome size) was observed in the variety Shatamangala, i.e., 6.623 pg/2C (3.238 Gb/1C) followed by Mohitnagar 6.557 pg/2C (3.206 Gb/1C) and the lowest DNA content (genome size) was recorded from the variety Kahikuchi, 6.340 pg/2C (3.100 Gb/1C).

Among the arecanut varieties developed from exotic germplasm, the mean DNA content (genome size) was 6.467 pg/2C (3.162 Gb/1C). The DNA content was varied from 6.025 pg/2C (2.946 Gb/1C) to 6.710 pg/2C (3.281 Gb/1C). The highest DNA content was observed in the variety Swarnamangala, 6.710 pg/2C (3.281 Gb/1C), followed by Sumangala 6.695 pg/2C (3.273 Gb/1C) and the lowest DNA content was recorded from the variety Sreemangala, 6.025 pg/2C (2.946 Gb/1C).

As reports on estimation of genome size in arecanut using flow cytometry analysis are not available, this study appears to be the first report in arecanut. The results of this study indicate the potential of using flow cytometry for studying genome diversity in arecanut. The results from the present investigation can be applied for further analysis of DNA content/genome size in large number of arecanut varieties/germplasm, wild species, related genera, inter-specific hybrids and rare arecanut mutant/abnormal

types to better understand the intra-specific, inter-specific variation in the crop and arrive at meaningful conclusion for better utilization in arecanut improvement programmes. The possibility of using this for varietal discrimination/identification of true to type planting material also needs to be explored.

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RESEARCH ARTICLE

An Overlooked Weed “Purpletop Vervain” *Verbena incompta* P. W. Michael (Verbenaceae) Forms a New Record to the Flora of India

Anjula Pandey, Nivedhitha Sivaraman, Kanakasabapathi Pradheep, Pavan K. Malav*, Rita Gupta and Sudhir P. Ahlawat

Abstract

Verbena incompta P. W. Michael (Verbenaceae) an invasive species from Brazil is reported here as a new record for the flora of India. This species was observed growing as weeds along agricultural fields in western Uttar Pradesh, India and profusely flowering and fruiting with high seed number. It is morphologically similar to *V. brasiliensis* Vell. and *V. bonariensis* L. but clearly distinct from the latter species in leaf and inflorescence characters. The present account provides detailed botanical description and comparative account between three allied species, namely *Verbena incompta* P.W. Michael, *V. brasiliensis* Vell. and *V. bonariensis* L. along with identification key. Weed risk assessment indicated *V. incompta* as a “serious weed” with high invasive potential.

Keywords: *Verbena incompta*, Exotic species, Weed risk assessment, India, Invasive potential.

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Introduction

The biggest threat to biodiversity from various anthropocentric activities is associated with many invasive plant and animal species. The appearance of exotic weed plants species in any new geographical areas is linked to the introduction. They occupy natural wastelands and habitats that are considered suitable sites for invasive weeds. Among many weed taxa, the ones belonging to the genus *Verbena* L. are globally known to invade or naturalize newer habitats.

The Indian region includes three wild species of *Verbena*, *V. bonariensis* L., *V. officinalis* L., *V. rigida* Spreng and nine cultivated species viz. *V. aubletia* Jacq., *V. chamaedryfolia* Juss., *V. hastata* L., *V. incisa* Hk., *V. peruviana* Druce, *V. phlogiflora* Cham., *V. platensis* Spreng., *V. tenuisecta* Briq. and *V. hybrid* Voss occasionally occurring in India (Rajendran and Daniel, 2002). *V. officinalis*, *V. bonariensis* and *V. rigida* are reported for their medicinal value in India (Ambasta, 1986). Cultivated species *V. bonariensis* is reported to run as an escape in states (districts in parenthesis) Tamil Nadu (Nilgiris), Himachal Pradesh (in Kangda, Madi); and *V. officinalis* in Himachal Pradesh (Cahmba, Kangda, Kinnaur, Kullu) (Hooker, 1885; Collett, 1902; Gamble, 1924; Chowdhury and Wadhwa, 1984).

The present paper describes the occurrence of *Verbena incompta* P.W. Michael, observed during an exploration undertaken in parts of Uttar Pradesh and adjoining areas of Haryana during October, 2020, forming a new record for India. Distinctive characters from existing *Verbena* species that are reported from India have confirmed its status as “new taxon” for the region. An identification

key is provided in light of a comparative account with three closely related non-native species of *Verbena*. Description of *Verbena incompta* with morphology, distribution, habitat details and identification key are provided. Invasive behavior through weed risk analysis is discussed in this paper to support concern for the weedy potential of *Verbena incompta* in India.

Materials and Methods

A weed species was recorded during exploration in parts of Uttar Pradesh and adjoining areas of Haryana in October, 2020. Upon critical examination of the species, comparing with three taxa occurring in India and other exotic allied species, and validating the information available in Indian floras, the taxon was confirmed as species not recorded in India.

Observations were made during visit to the site in 2020, followed by a revisit to the site in 2021 to ascertain the population spread. The virtual herbarium of BM, E, K, P, PE and NHCP (Thiers, 2019) and the global databases viz. National Gene Bank (NGB), Global Biodiversity Information Facility (GBIF), Germplasm Resources Information Network (GRIN), Kew Catalogue, etc. were studied for eco-geographic records. A comparative study was also undertaken using herbarium specimens of allied taxa available in the CNH, DD and National Herbarium of Cultivated Plants (NHCP)] and the online resources and supplemented with relevant literature to establish it as new record for India. Voucher herbarium specimens (HS23146, 23147, 23150) were prepared as per standard methods and deposited in the National Herbarium of Cultivated Plants (NHCP), ICAR-NBPGR, New Delhi.

Weed risk assessment was based on a standard format as prescribed by Singh *et al.* (2013). This tool was subjected to provide status of the species invasiveness based on defined biological and ecological parameters through a questionnaire containing 49 questions. The resultant question-based scoring was assessed to define the “weediness status” level. As per data on field assessment, the response to the questions generated a numerical score with a positive correlation to weediness (Annexure 1).

Results and Discussion

Distribution and Habitat Details

The genus *Verbena* (family Verbenaceae) is represented by 250 species which are mainly spread in sub-tropical and tropical countries of America, also in Europe, Asia, Africa and Australia (Yeo, 1990; Rajendran and Daniel, 2002).

Verbena incompta P.W. Michael locally called “Purpletop Vervain” is presumed to be a taxon of South American origin (Brazil, Argentina, Paraguay). It is widely distributed in sub-tropical and warmer temperate parts of the world; it grows as an invasive weed in grasslands, agricultural fallow lands, range lands, urban areas preferring humid

habitats (Mikeladze *et al.*, 2017). *V. incompta* is reported to be naturalized in Italy and Spain, invasive weed in Belgium (Verloove, 2006; 2011) and a declared noxious weed in many temperate and subtropical areas worldwide. Its adaptation for drought and heat tolerance and predominantly on disturbed habitats along roadsides, new forest plantations, forest edges, dry or moist river beds, ditches, road verges, and more rarely in ruderal make it the most successful taxon as a weed.

V. incompta was described as a species new to science (Michael, 1995). It occurs in parts of Europe and elsewhere along with other species, *V. bonariensis* and/or *V. brasiliensis*. Due to its great affinity with the related species, *V. brasiliensis* Vell. and *V. bonariensis* L. there are several identity-related issues (Perry, 1933; Verloove, 2011). *V. incompta*, much more reputed as a weed than *V. bonariensis* and without any ornamental value. The remarkable concentration of *V. incompta* around agricultural areas is accounted for inadvertent introduction along with foreign seeds, soil, and other objects. *V. incompta* has been possibly confused with *V. bonariensis* in southern Europe where the latter is claimed as a naturalized alien (Verloove, 2011; Gleason and Cronquist, 1991; Liendo *et al.*, 2016).

Three species occurring in India, namely *V. officinalis*, *V. bonariensis*, and *V. rigida* did not match with the presently reported taxon. After critical morphological observations, comparison with the herbarium specimens and validation from relevant literature, the identity of the species was confirmed as *Verbena incompta*.

V. incompta very clearly differs from other *Verbena* species in India. *V. bonariensis* is reported from Himachal Pradesh and Uttarakhand and is distinguished by the erect stem, sessile and glandular leaves, longer inflorescence having not showy corolla tubes and shorter mericarps. The second species namely, *V. officinalis* L. has an erect stem, petiolate ovate-lanceolate, papery leaves, margin variously toothed, acute apex, inflorescence with flowers lax in slender panicle, showy blue-pink corollas. This species occurs widely across different states, Punjab, Uttar Pradesh, Andhra Pradesh, Bihar, Himachal Pradesh, J&K, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram and Nagaland. The third species, *V. rigida* has a rhizomatous creeping stem, leaves oblong-lanceolate, adnate at base, coarsely serrate margins, acute apex, spikes terminal, corolla pink, dark purple-blue-violet (is an escape in Karnataka and Tamil Nadu).

New Distribution Record

The newly reported species *Verbena incompta* was distributed along with natural vegetation in disturbed habitat in village Barauli, district Baghpat, Uttar Pradesh, India. The authors recorded a population in present locality in village Barauli, Baghpat district, Uttar Pradesh, India, during field survey and germplasm collection of crop wild relatives and potential taxa. Plant populations were densely

spread across wider habitats ranging from dry agricultural fellow land, ditches along roadsides, wet land along the field margin. Over 6 to 7 populations in around 200 m area were observed to flower and seed ripening simultaneously. The plants were not located beyond the reported area, indicating its introduction as a weed in recent past.

The global germplasm resource databases (NGB, GBIF, GRIN/USDA) did not report this species from Uttar Pradesh, India. Recent report on the occurrence of *Verbena brasiliensis* from Himachal Pradesh (BSI 2020-21) was found to be closer to *V. incompta*. (<https://sites.google.com/site/efloraofindia/species/m---z/v/verbenaceae/verbena/verbena-brasiliensis>). This may indicate that this taxon has been largely overlooked due to similarity with other taxa. The present report is new distributional record of *Verbena incompta* in India from Uttar Pradesh.

Botanical Description

Erect herb grows as annual to perennial, 50 to 200 cm tall. Stem sharply 4-angled, hispid on the angles. Leaves simple, sessile, opposite, decussate, semi-amplexicaul-subauriculate, lanceolate, up to 15 x 3 cm, sharply and irregularly serrate, entire towards the base, rugose-scabrous on both surfaces. The inflorescence is corymbose to spicate. Spikes sub-cylindrical, 3 to 10 in number, up to 5 to 7 cm long and up to 5 mm wide at maturity. Flowers small,

and numerous, opening together in a circle immediately below the apex of the spike. Floral bracts ovate-lanceolate, 2 to 3 mm long, glandular or with minute and very sparse stalked glands. Calyx 5-angled, pubescent, 5-toothed at apex. Corolla small mauve/blue-purple, limb c.2.75–3.75 mm wide, tube 2.75 to 3.25 mm long, slightly curved and hardly exceeding the calyx tube. Upper anthers are inserted above the middle of the corolla tube. Fruit included in the persistent calyx tube, glabrous, splitting into 4 cylindrical-oblong brown mericarps, mericarps-1.3 to 1.5 mm long, ribbed on the outside, weakly reticulate.

Location

Baghpat district, Uttar Pradesh): collector no. ANP-7-1, village Barauli, Baghpat district, Uttar Pradesh, India (with average altitude 28.59.821; 77.13.457 and 29.04.159; 77.13.570); October 6, 2020.

Ecology

Other wild taxa *Cenchrus ciliaris* L., *Cynodon dactylon* (L.) Pers., and *Chenopodium album* L., *Achyranthus aspera* L. co-occur in the open grassy fallow land.

Flowering and Fruiting

October-November (present study); October-January (Michel, 1995).



Figure 1: *Verbena incompta*: (top row L-R) plant population; newly emerging plants with typical leaves; (bottom row L-R) flower showing close-up; mature plants with dried inflorescence (inset).

Use

The species was unfamiliar to local people; no use of this species was noted in the area of collection.

Notes: Plants were noted with compact elongated spikes with sprinkling small mauve/ blue-purple flowers. The plant populations were noted in vegetative, flowering, seeding, and shattering stages simultaneously and dried spikes amongst the fresh plant growth (Figure 1).

Identity Confusion with Other Taxa

The genus *Verbena* includes reputed environmental weeds such as *V. brasiliensis* Vell. And also popular ornamentals such as *V. bonariensis* L. and *V. rigida* Spreng. Despite the existence of a recent revisionary works, the taxonomy and nomenclature of the *V. bonariensis* complex remain unclear (Munir, 2002; Michael, 2008; Nesom 2010).

V. incompta resembles very much with *V. bonariensis* as well as *V. brasiliensis*. With the former it has the sessile-amplexicaul leaves in common, whereas the characteristics of inconspicuous corollas, long spike inflorescence and small mericarps are shared with *V. brasiliensis* (Verloove, 2011). Like *V. brasiliensis*, it lacks ornamental value due to inconspicuous corollas (Yeo, 1990). The corollas of *V. brasiliensis* and *V. incompta* are only slightly narrower than those in *V. bonariensis* (c. 2.75 to 3.75 mm versus 4.25 to 5.5 mm (Yeo, 1990), but they are hardly expanded from the calyx tube and most become even virtually invisible in herbarium specimens. The ripe mericarps of *V. incompta* and *V. brasiliensis* are smaller than in *V. bonariensis*. Three species, *V. incompta* and *V. brasiliensis* and *V. bonariensis* can be delineated on the basis of micro-morphological characters; *V. incompta* and *V. brasiliensis* have minute stalked glands on calyx tube and absent on pedicels (or very sparse) whereas *V. bonariensis* has abundant glands (Yeo, 1990; Michael, 1995; 2008). *Verbena incompta* appear to be intermediate between *V. bonariensis* and *V. brasiliensis* (Verloove, 2011). The identification key for three *Verbena* species is presented below:

1. Leaves distinctly tapering to base ***V. brasiliensis***
1. Leaves not tapering to base
2. Corolla not showy, hardly exerted from calyx tube; inflorescence spikes slender and sub-cylindrical, usually up to 5 to 7 cm long ***V. incompta***
2. Corolla showy, well exerted from calyx tube; inflorescence spikes usually short and thick, frequently congested at maturity, rarely more than 1.5 cm long ***V. bonariensis***

Weed Risk Analysis

Many garden plants of exotic origin pose a high risk of naturalization and potential as a weed in the area of cultivation (Groves *et al.*, 2005; Pandey *et al.*, 2018). Species of *Verbena* have reports of invasiveness in many countries (<http://fnai.org/Invasives/FNAI.pdf>) coupled with

adaptation to diverse soil types ranging from wetland to drought affected regions that poses risk of establishment as naturalization. The plant traits such as luxuriant growth, plants remaining green all through the year, high-fruit bearing and high seed number/fruit, perennating root stocks, no natural predators (not eaten by animals) and dispersal through movement of animals/soil to influence fast spread of the species have favoured invasiveness in the newer areas. The species may gradually dominate and completely replace other natural/native vegetation.

V. incompta is a declared noxious weed in many temperate and subtropical areas worldwide (GISD, 2007; Verloove, 2011). Keeping this in view, weed risk assessment was made based on standard procedure (Singh *et al.*, 2013). A question-based scoring of '18' for this species revealed its potential as "serious weed" in cultivable/agricultural land (Annexure 1). Appropriate and timely monitoring for its future spread was indicated.

Conclusions

This is the first report on occurrence of *V. incompta* recorded by the authors in the territory of village Barauli, district Baghpat, Uttar Pradesh in India. The present study adds to our understanding on new distributional record and weedy potential of the species. Besides, visual observations on its spread as a weed in areas near cultivation sites and weed risk analysis has raised the risk of fast invasiveness through escaping from areas of naturalization. Monitoring of *V. incompta* population in the present locality and spread elsewhere seems timely.

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Annexure 1: Weed risk assessment questionnaire Answer yes (y) or no (n), or don't know (leave blank or?), unless otherwise indicated

Common name:		Botanical name: <i>Verbena incompta</i> P.W. Michael			Outcome:	Reject
Family name:		Purpletop Vervain				
		Verbenaceae	Your name: Dr. Anjula Pandey			
History/Biogeography						
A	1	Domestication	1.01	Is the species highly domesticated. If answer is 'no' go to question 2.01		n
C		Cultivation	1.02	Has the species become naturalized where grown		
C			1.03	Does the species have weedy races		
	2	Climate and	2.01	Species suited to Indian climates (0-low; 1-intermediate; 2-high)	y; 1	
		Distribution	2.02	Quality of climate match data (0-low; 1-intermediate; 2-high)	0	
C			2.03	Broad climate suitability		y
C			2.04	Native or naturalized in regions with extended dry periods		y
			2.05	Does the species have a history of repeated introductions outside its natural range		?
C	2	Weed	3.01	Naturalized beyond native range		y
E		elsewhere	3.02	Garden/amenity/disturbance weed		y
A			3.03	Weed of agriculture/horticulture/forestry		y
E			3.04	Environmental weed		
			3.05	Congeneric weed		
Biology/Ecology						
A	4	Undesirable	4.01	Produces spines, thorns or burrs		n
C		traits	4.02	Allelopathic		
C			4.03	Parasitic		

A			4.04	Unpalatable to grazing animals	
C			4.05	Toxic to animals	
C			4.06	Host for recognised pests and pathogens	
C			4.07	Causes allergies or is otherwise toxic to humans	
E			4.08	Creates a fire hazard in natural ecosystems	
E			4.09	Is a shade tolerant plant at some stage of its life cycle	
E			4.10	Grows on infertile soils	y
E			4.11	Climbing or smothering growth habit	y
E			4.12	Forms dense thickets	y
E	5	Plant type	5.01	Aquatic	
C			5.02	Grass	
E			5.03	Nitrogen fixing woody plant	
C			5.04	Geotype	y
C	6	Reproduction	6.01	Evidence of substantial reproductive failure in native habitat	
C			6.02	Produces viable seed	y
C			6.03	Hybridises naturally	
C			6.04	Self-fertilisation	
C			6.05	Requires specialist pollinators	
C			6.06	Reproduction by vegetative propagation	y
C			6.07	Minimum generative time (years)	y
A	7	Dispersal	7.01	Propagules likely to be dispersed unintentionally	y
C		mechanisms	7.02	Propagules dispersed intentionally by people	y
A			7.03	Propagules likely to disperse as a produce contaminant	y
C			7.04	Propagules adapted to wind dispersal	
E			7.05	Propagules have dormancy	
E			7.06	Propagules bird dispersed	
C			7.07	Propagules dispersed by other animals (externally)	y
C			7.08	Propagules dispersed by other animals (internally)	
C	8	Persistence	8.01	Prolific seed production	y
A		attributes	8.02	Evidence that a persistent propagule bank is formed (>1 yr)	
A			8.03	Well controlled by herbicides	
C			8.04	Tolerates or benefits from mutilation, cultivation or fire	
E			8.05	Effective natural enemies present in India	

A=agricultural, E=environmental, C=combined

Score: 18

SHORT REPORTS

Current Status of Plant Genetic Resources Conservation and Use in the National Genebank, Nigeria

Ibidunni S. Adetiloye*, Sunday E. Aladele, Anthony U. Okere and Adam Olosunde

Abstract

The aim of this paper is to expound the current status of the conservation and management system of plant genetic resources (PGRs) in the National Genebank of Nigeria. National Centre for Genetic Resources and Biotechnology (NACGRAB) created in 1987 is referred to as National Genebank. It holds one of the most comprehensive collections of PGR in the form of seed, plantlets, live plants in Nigeria and provides a major contribution to the prevention of Genetic Resources erosion.

The genebank currently conserved about 10,200 accessions of more than 25 crops species viz 5,464 accessions of cereals, 2,193 accessions of pulses/legumes, 556 accessions of oilseeds, 1,674 accessions of vegetables while about 160 accessions of various tree species are conserved *ex-situ*. Diversity mapping, characterization and evaluation of PGRs, duplicate identification, diversity study, pre-breeding and landrace enhancement are the major research works of NACGRAB, and its management strategies such as conservation method, types, characterization, and evaluation are very essential for strengthening utilization of the PGRs.

Keywords: Conservation, Characterization and evaluation, *Ex-situ*, Genebank, PGRs, Utilization.

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Introduction

Nigeria is a country that is physically and ecologically diverse; and greatly endowed with natural resources. The country can be divided into 3 distinct ecological regions: a humid forest region, a sub-humid region with highland and a semi-arid region with an annual rainfall of about 250 to 3000 mm in Sahelian north and southern coastal areas, respectively (FAO, 2008). There are 7,895 plant species from 338 families and 2,215 genera that have been identified in Nigeria (FMEnv, 2006). However, about 0.4 and 8.5% of the plant species are said to be threatened and endangered according to the International Union for Conservation of Nature (IUCN) list of the threatened species.

Nigeria, rich in plant genetic resources and their wild relatives in their natural habitats, is endowed with rich and is the epicenter of diversity for many crops/taxa such as cowpea, West Africa okra, West African rice, yams, bambara groundnut, Africa yam bean and wing bean (FMEnv, 2006).

PGRs Management System in Nigeria

Plant Genetic Resources (PGRs) or germplasm refers to all materials that can be used for improvement of cultivated plant species (Becker, 1993). According to the International convention for biodiversity, genetic resources is defined as all living materials that include genes of present and potential value for human use and needs (IPGRI, 1993).

Genebanks (*ex-situ* conservation) were created in the mid-twentieth century to conserve indigenous cultivated biodiversity

when modern varieties replaced landraces. This idea was accepted generally as a necessary step to safeguard the future (Van de Wouw *et al.*, 2009 and Khoury *et al.*, 2014). Genebanks were created to preserve genetic material for immediate use and posterity (Fowler and Hodgkin, 2004), either directly or as genetic material in breeding programs to face challenging situations in environmental conditions or societal needs.

The national centre for genetic resources and biotechnology was established in 1987 with a mandate to conduct research, gather information/data and disseminate technological information on matters relating to genetic resources, conservation, utilization and biotechnology applications. It is the focal point for Plant genetic resources conservation and maintenance in Nigeria and is thereby referred to as the National genebank. In line with her mandates, it has four major departments/units, namely, Plant Genetic Resources Department (Seed Genebank and Field genebank), Animal Genetic Resources, and biotechnology (Tissue culture and molecular biology units). In addition, the Centre also has 3 Zonal Stations Research Farms located in different geo-political zones viz: Bagauda in Kano state; Badeggi in Niger state and Benin in Edo state.

National Centre for Genetic Resources and Biotechnology (NACGRAB) has adopted various strategies in order to manage the rich genetic resources available in the country. The strategies include: *Ex-situ* conservation which comprises of seed genebank, *in-vitro* bank, field genebank and medicinal garden. Types and groups of germplasm available in the genebank for conservation are as follows: Landraces, modern/improved varieties, obsolete varieties, breeding lines, inbred lines, exotic lines, and wild relatives. The PGRs are also grouped based on economic values, such as cereals, pulses, vegetables, fruits, fibers, oil crops, spices etc. Based on the biology of the seeds/germplasm, all germplasm are divided into: orthodox seeds, recalcitrant seeds and vegetatively propagated plants. The centre also adopted geographical information system (GIS) application, characterization, regeneration and evaluation of PGRs, identification of duplicates, diversity study, and pre-breeding, among others.

The facilities available in the center include:

- Medium-term storage (initially Long term storage with vacuumed sealed material used for base collection but has been converted to medium storage due to the present condition the genebank) with 2 to 10°C and 15% RH. This facility can store for 15 to 20 years;
- Short-term storage (for active collection) with 20–25°C and 15% RH. It can store for 2 to 5 years for orthodox seeds;
- *In-vitro*/tissue culture laboratory: for *in-vitro* conservation of recalcitrant, apomictics and vegetatively propagated plants.

- Seed testing and processing laboratory,
- Seed health laboratory
- Field genebank: for conservation of various tree spp.
- Molecular laboratory: for genetic analysis at DNA level e.g., DNA extraction, PCR amplification etc.
- Database management: to document all the information pertaining to the conserved germplasm.

Exploration, Collection, Conservation and Utilization of Germplasms

NACGRAB has been collecting/exploring PGRs from different parts of the country since 1987 either alone or in collaboration with other institutes. More than 10,200 accessions of indigenous cultivars and wild relatives have already been collected through over 25 crop-specific and region-specific explorations (Table 1) and they are being maintained at the centre's storage facilities. These represent wide variability in crops like maize, sorghum, rice, wild vigna, okra, chochorus, amaranthus, and pearl millet. The areas already explored include the North-East, North-West, North-Central, South-West, and South-East regions of Nigeria. However, some of the preserved germplasm is constantly lost due to poor storage conditions due to power outages or unstable power supply. However, gap analysis is regularly done before embarking on explorations in order to explore some of the old site where the lost germplasm were collected. Under *ex-situ* conservation, the genebank holds about 10,200 accessions of more than 65 crop species with their passport data (collected through over 25 crop specific and region-specific explorations). Among them are, 5,464 accessions of cereals, 2,193 accessions of pulses/legumes, 556 accessions of oilseeds, 1,674 accessions of vegetables while about 160 accessions of various tree crop species are being conserved in the field genebank facility of the centre (Figure 1).

A total of 3,266 accessions of 26 crops species representing about 30% of total germplasm maintained in the facility were regenerated during 2015-2019 in Ibadan

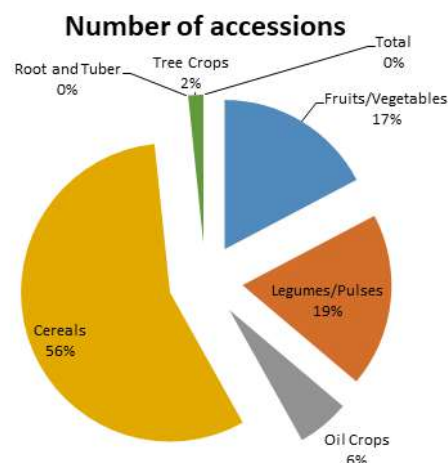


Figure 1: Crop class and number of accessions in storage.

Table 1: Number of crop species and accessions in storage at NACGRAB's genebank as at 2020

S. No.	Crop	Genus	Species	Seed Genebank	Field Genebank	In-vitro Genebank
1	Amaranthus	<i>Amaranthus</i>	<i>Hybridus</i>	252		
2	Celosia	<i>Celosia</i>	<i>Argentia</i>	248		
3	African Spinach	<i>Basella</i>	<i>Alba</i>	12		
4	Cabbage	<i>Brassica</i>	<i>Oleraceae</i>	3		
5	Roselle	<i>Hibiscus</i>	<i>Spp</i>	12		
6	Basil	<i>Ocimum</i>	<i>Basilicum</i>	6		
7	Saint Leave	<i>Ocimum</i>	<i>Graticinum</i>	3		
8	Bambara Nut	<i>Vigna</i>	<i>Subterra</i>	42		
9	Soybean	<i>Glycine</i>	<i>Max</i>	424		
10	Lima/Butter Bean			92		
11	Phaseolus	<i>Phaseolus</i>	<i>Spp</i>	52		
12	Pigeon Pea	<i>Cajanus</i>	<i>Cajan</i>	50		
13	Sword Bean	<i>Canavalia</i>	<i>Gladiata</i>	3		
14	African Yam Bean	<i>Sphenostylis</i>	<i>Stenocarpa</i>	100		
15	Groundnut	<i>Arachis</i>	<i>Hypogea</i>	368		
16	Wild Vigna	<i>Wild Vigna</i>	<i>Species</i>	1062		
17	Pumpkin	<i>Curcubita</i>	<i>Spp</i>	27		
18	Cucumber	<i>Cucumis</i>	<i>Sativus</i>	6		
19	Melon	<i>Cucumis</i>	<i>Spp</i>	444		
20	Egg Plant	<i>Solanum</i>	<i>Spp</i>	114		
21	Pepper	<i>Capsicum</i>	<i>Spp</i>	214		
22	Tomato	<i>Solanum</i>	<i>Lycopersicum</i>	144		
23	Rice	<i>Oryza</i>	<i>Sativa</i>	492		
24	Millet	<i>Pennisetum</i>	<i>Spp</i>	1467		
25	Wheat	<i>Triticum</i>	<i>Aestivum</i>	212		
26	Sorghum	<i>Sorghum</i>	<i>Bicolor</i>	1808		
27	Maize	<i>Zea</i>	<i>Mays</i>	1375		
28	Fonio/Acha	<i>Digitaria</i>	<i>Exillis</i>	110		
29	Onion	<i>Allium</i>	<i>Cepa</i>	9		
30	Okra	<i>Abelmoscus</i>	<i>Esculentus</i>	506		
31	Corchorus	<i>Corchorus</i>	<i>Olitorius</i>	118		
32	African Apple	<i>Chrisophyllum</i>	<i>Albidum</i>	3		
33	Sesame	<i>Sesamum</i>	<i>Indicum</i>	97		
34	Morogbo (Sesame Vegetable)	<i>Sesamum</i>	<i>Indicum</i>	12		
35	Castor Seed	<i>Ricinus</i>	<i>Communis</i>	3		
36	Cotton	<i>Gossypium</i>	<i>Hirsutum</i>	12		
	TOTAL SEED			9902		
37	Acacia	<i>Acacia</i>	<i>Albida</i>		1	
38	Acacia	<i>Acacia</i>	<i>Nodosa</i>		8	
39	Acacia	<i>Acacia</i>	<i>Biflora</i>		7	
40	Baobab	<i>Adansonia</i>	<i>Digitata</i>		4	
41	Azvelia	<i>Azvelia</i>	<i>Africana</i>		2	
42	Agava	<i>Agava</i>	<i>Spp</i>		5	

43	Albizia	Albizia	<i>Odoratisswima</i>	20
44	Albizia	Albizia	<i>Saman</i>	3
45	Albizia	Albizia	<i>Lebbeck</i>	16
46	Alstonia	Alstonia	<i>Boonei</i>	2
47	Cashew	<i>Anacardium</i>	<i>Occidentale</i>	7
48	Anogeisus	Anogeisus	<i>Leocardpus</i>	2
49	Sweet Sops	<i>Anona</i>	<i>Squamosal</i>	3
50	Anthonotha	Anthonotha	<i>Macrophylla</i>	6
51	Sour Sop	<i>Anonas</i>	<i>Muricata</i>	2
52	Bread Fruit	<i>Artocarpus</i>	<i>Altilis</i>	8
53	Avocado Pear	<i>Persea</i>	<i>Americana</i>	3
54	Neem	<i>Azadiracthta</i>	<i>Indica</i>	37
55	Bauhina	Bauhina	<i>Monandra</i>	5
56	Bixa	Bixa	<i>Orellana</i>	5
57	Ackee Apple	<i>Blighia</i>	<i>Sapida</i>	12
	TOTAL			157
58	Plantain/Banana	<i>Musa</i>	<i>Spp</i>	2
59	Pineapple	<i>Ananas</i>	<i>Comosus</i>	3
60	White Yam	<i>Dioscorea</i>	<i>Rotundata</i>	3
61	Water Yam	<i>Dioscorea</i>	<i>Alata</i>	1
62	Ginger	<i>Zingiber</i>	<i>Officinale</i>	2
63	Bitter Leaf	<i>Vernonia</i>	<i>Spp</i>	1
64	Passion Fruit	<i>Passiflora</i>	<i>Edulis</i>	1
65	Cocoyam	<i>Colocasia</i>	<i>Esculenta</i>	2
66	Eucalyptus	<i>Eucalyptus</i>	<i>Spp</i>	1
Total				16
Grand Total				10,075

(Oyo state), Bagauda (Kano state) and Badeggi (Niger State) stations (Table 2). Moreover, a total of 310 accessions of 6 crop species were characterized and evaluated i.e., 100 accessions of maize, 50 accessions of rice, 20 accessions of cowpea, 40 accessions of okra, 40 accessions of Jute mallow and 60 accessions of tomato (Table 3).

One of the mandates of genebank is utilization by providing access to PGRs and databases. The genebank has distributed PGRs to national research institutes, students, scientists, breeders, hobby breeders and farmers majorly for utilization in crop improvement programmes. 9888 accessions of 40 different crop species were distributed to various users (Table 4).

Links and Collaboration with International Organizations (CGIAR), NARIs and Private Organizations

In order to fulfill its national mandate, the centre maintains links and collaboration with all National crop/Animal based institutes of the Agricultural Research Council of Nigeria (ARCN), Agricultural Universities and also have effective links with many crop based) since the inception of the

genebank in 1987. The Centre collaborates with CGIAR centres, including Genetic Resources Centre (GRC) housed in International Institutes under the CGIAR system, include Centre for Genetic Resources (CGR) housed in the International Institute for Tropical Agriculture (IITA), Ibadan managing the world plant genetic resources and ICRISAT works on the management of wheat, millet, pearl millet plant genetic resources. In addition, there are other NGOs involved in Agro biodiversity management e.g., Centre for Environment, Renewable Natural Resources Management, Research and Development (CENRAD), Justice Development Movement (JDPM), Oyo, Nigeria etc. and many other working on seed business. As part of mandates of NACGRAB in the conservation of safety duplicates of germplasm held by National Agricultural Research Institute (NARIs) in Nigeria, the under-listed germplasm were deposited at the National Gene bank.

Duplicates Samples Deposited at International Genebanks

In accordance with the provision of treaty of Conventional Biological Diversity of 1992 which mandated all genebanks

Table 2: Total number of accessions per crop species successfully regenerated at Ibadan from 2015 - 2019

S. No.	Type of Crops	Number of Accessions
1	Okra	215
2	Corchorus	153
3	Amaranthus	100
4	<i>Bassella alba</i> (Red And White)	17
5	Tomato	140
6	Melon	50
7	Pepper	82
8	Ife Bimpe Minor Legumes	10
9	Maize	36
10	Sorghum	100
11	Celosia	58
12	Yam	34
13	Rice	50
14	Snake Tomato	2
15	Roselle	5
16	Sesame	8
17	Groundnut	40
18	Soybean	20
19	Bambara Nut	18
20	Pearl Millet	240
21	Cowpea	59
22	African Yam Bean	6
23	Phaseolus	3
24	Pigeon Pea	10
25	Egg Plant	40
26	Wild Vigna	20
Total		1516

in the world to deposit duplicate samples in Svalbard Global Seed Vault, Norway. A total of 423 sorghum accessions, 176 accession of cowpea and 34 accessions of sesame were deposited in Svalbard Genebank, Norway (Annual Report, 2012). In collaboration with the Institute of Agricultural Research (IAR), Zaria, Lake Chad Research Institute (LCRI), Maiduguri and National Roots Crops Research Institute (NRCRI), Umudike supported by Global Crop Diversity Trust, additional 423 accessions of sorghum and 167 accession of pearl millet were deposited in ICRISAT Sahelian Centre, Niamey for safe keeping (Figure 2).

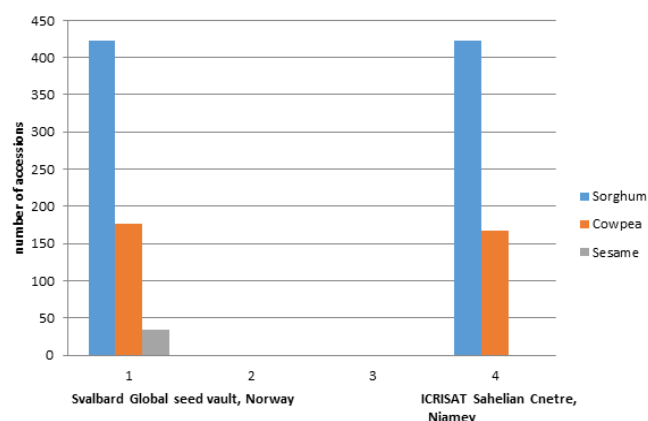
Documentation and Information

Significant advances have been made in terms of documentation and publicly making information on ex situ germplasm available. The genebank has upgraded from using the Ms Excel application for data management to using Genesys as an information management system. Passport data of more than 51 accessions of crop wild relatives (CWR)

Table 3: Accessions of crop characterized

S. No.	Crop spp	Number of accessions
1	Maize	100
2	Rice	50
3	Cowpea	20
4	Okra	40
5	Jute mallow	40
6	Tomato	60
7	Total	310

Safety duplicates of germplasm deposited at international genebanks

**Figure 2:** Four crop species safely duplicated in CGIAR banks.

and 4000 landraces were submitted to the *genesis* and FAO database (Figure 3). Figure 2 explained the number of accessions conserved in different facilities in NACGRAB. 98% of the total accessions which represent 9902 accessions are stored in the seed genebank while only 2% representing 157 accessions are in the field genebank. The figure revealed that only 16 accessions are stored *in-vitro*.

Links and Collaboration with International Organizations (CGIAR), NARIs and Private Organizations

In order to fulfil its National mandate, NACGRAB has a strong synergy and collaboration with international, regional and local organizations (Institutes and Universities that are crop based) since the inception of the genebank in 1987. The Centre collaborates with CGIAR centres, including Genetic Resources Centre (GRC) housed in International Institute for Tropical Agriculture (IITA), Ibadan managing the world plant genetic resources and ICRISAT working on the management of wheat, millet, and pearl millet plant genetic resources. In addition, The centre maintains a linkage with FAO for support to promote the conservation and sustainable use of PGRFA. In addition, there are other NGOs involved in Agro biodiversity management e.g. Centre for Environment, Renewable Natural Resources Management, Research and Development (CENRAD), Justice Development Movement

Table 4: Seed Distribution From 2010-2019

<i>Crop spp</i>	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Amaranth	113	44	136		69	195	127	114	82	204	1084
African yam bean			5		2	12	25	2	13	9	68
Acha					1	2	2		2	19	26
African spinach								4	1	2	7
Basil											
Bambara nut	36				5	30	5	7			83
Butter bean						5	3				8
Black bean											
Celosia			11		16	40	15	57	58	96	293
Castor											
Cowpea	10	42	98		109	143	97	123	71	36	729
Cabbage											
Cotton					5	7			1		13
Cucumber									1	3	4
Egg plant			34		36	56	45	32	50	42	295
Eleusine									1		1
Groundnut	47		14		21	11	17	18	21	19	168
Jute mallow	20	29	43		71	45	61	55	101	168	593
Kenaf					8	6	12	36	22	15	87
Lima bean			3				5				8
Millet		7	2		5	25	11	3	41	41	135
Melon	20	34	30		37	42	32	4	14	45	258
Maize	145	39	22		14	103	179	136	59	28	725
Okra	100	66	96		145	89	73	166	155	242	1132
Onion						1			3		4
Pepper	40	31	25		31	60	66	104	58	145	560
Pumpkin	11					5			1		17
Pigeon pea	3				1	39	14	7	25		89
Phaseolus						2			1		3
Roselle					5	1		2	1	3	12
Rice					12	51	46	71	18	107	305
Sesame	5		42		45	42	112	32	76	9	363
Sword bean											
Sorghum	36	165	178		62	249	96	56	154	91	1087
Soybean	6		15		28	56	19	39	28	89	280
Sunflower						10			1		11
Tomato	52	30	61		194	137	286	155	98	151	1164
Water melon										4	4
Wheat					4	2		6	10	9	31
Wild vigna					140	91			10		241
Total	644	487	815		1066	1557	1336	1229	1177	1577	9888

(JDPM), both in Oyo state, Nigeria and many other working on seed business. With support of WAAPP-Nigeria, the genebank rehabilitated

the cold storage facilities and kept it functional during phase 1 of the project from 2013-2015. A technical consultant was also hired to ensure the genebank work perfectly at all

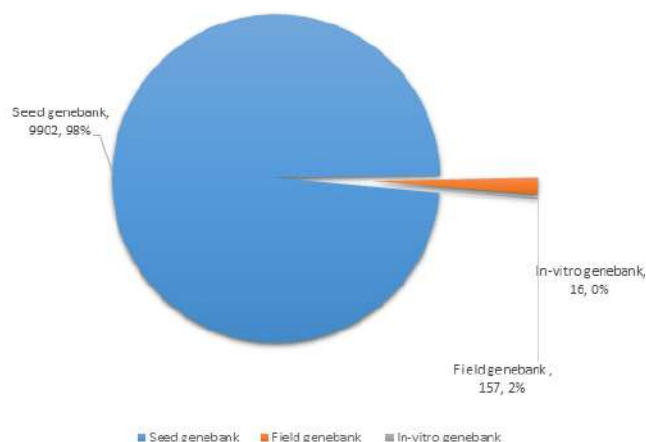


Figure 3: Percentage of crop accessions in different storage type.

times. The International Institute of Tropical Agriculture in Ibadan has been a major partner for NACGRAB. Recently, IITA and the centre jointly sampled Kersting's groundnut from Oyo, Ondo and Ekiti farms in October 2018, and groundnut and African Yam bean in January-February 2019.

The Global Crop Diversity Trust (GCDT), through the Royal Kew Botanical garden, in the U.K funded the collection of wild relatives of crops which includes: rice, garden egg, bambara groundnut, millet, and wild *Vigna*. Two hundred and six accessions of 20 species of wild relatives of crops. The materials collected with their herbarium specimen were processed at the NACGRAB and deposited in the gene banks of NACGRAB and Royal Kew Botanical garden for further classification and characterization. The GCDT through the crop wild relative project also provides support to NACGRAB to strengthen its genebank information systems. This project upgrades the computer hardware equipment for document management systems.

A research project on Wild *Vigna*, funded by the GCDT through the International Institute for Tropical Agriculture (IITA) was carried out in NACGRAB for two years. In 2017, (174) wild *vigna* lines were evaluated for seedling stage drought, the experiment identified thirty of the wild *vigna* accessions to be highly tolerant to seedling stage drought. The result obtained prompted the second stage of the experiments in 2018, out of the 162 accessions screened for seedling stage drought, 68 accessions were selected as tolerant to seedling stage drought based on their level of recovery after drought.

Crop Research Institute for the semi-arid tropics (ICRISAT) in the year 2017 and 2019. The germplasm collection activity was an extensive exploration activity designed to collect maximum diversity of different crops, some of which were on the verge of extinction and located in difficult terrain, hostile environments and harsh conditions. Major crops of focus during the collection activity included: sorghum, pearl millet and groundnut which have high economic importance and are tagged worldwide as crop for the future. In 2017, a total of 144 accessions were collected; in 2019, a total of 223 accessions were collected. The centre has also successfully supervised the collection and regeneration

of four crops, cowpea, sorghum, pearl millet and yam. The project was funded by the Global Crop Diversity Trust and had 3 Research Institutes, the Institute of Agricultural Research, Samaru (IAR), Lake Chad Research Institute (LCRI) and National Root Crops Research Institute (NRCRI) as collaborators. As part of the requirement of the project, safety duplicate of the materials collected have been sent to Svalbard Global Seed Vault and ICRISAT Sahelian centre, Niamey for safe keeping.

Training

The centre also impart training in all facets of PGR activities. It has conducted short term training at the national level on tissue culture molecular techniques. It has also trained several students from Universities, Polytechnic and colleges of agriculture on SIWES and industrial training. PhD students from different universities within the country and outside have also been regularly made use of the centres facilities for their experiments/research with an emphasis of training such students with a view to producing more specialists in field of plant genetic resources management. The centre has also been relevant in information dissemination on PGR activities in Nigeria. It published annual reports, newsletters (quarterly) and the National Varietal Release catalogue. The centre has well over 100 research papers, reports, catalogues, proceedings of seminar/symposia, etc. with focus on conservation and management of plant genetic resources research.

Many of the centre's scientists and technologists have received training both at national and international levels on different aspects of plant genetic resources conservation and utilization.

Gaps and Challenges Facing PGR Management in Nigeria

Technical Know-how

Crop wild relatives are related to our food crop plants and contain useful diversity for adapting food crops to climate change. Ironically, little effort and priority has been given to this class of germplasm. Technical capacity for management (evaluating and characterizing) of CWR is still lacking as there are few personnel with technical knowledge on collecting and managing CWR in the country.

Limited Financial Resources/Fund

Exploration, collection and characterization of PGRs needs a lot of funds but due to limited financial resources, collection, characterization, seed health, and regeneration activities are not effective and efficient.

Farmer's Unwillingness to Provide Information

During collections and exploration of germplasm, farmers don't always give enough information/knowledge associated with PGR. Also, they don't provide enough seed sample that warrants diversity within a landrace.

Lack of Infrastructures

The two facilities for germplasm storage (Long term and short-term) has been in existence since the inception of the genbank in 1987. Due to long-term of use, they are no longer efficient especially the long-term storage room. In addition, epileptic power supply also pose a problem to the efficiency of the cold room and threatens the longevity of the germplasms.

Gaps in PGRs Management among NARIs, NGOs and Private Organizations

Weak coordination existed among NARIs, NGOs and private organizations as regards germplasm management, exchange and information sharing. There are situations whereby these organization are not willing to provide information and samples of germplasm in their custody to the National genebank.

Assessment Genetic Erosion and Community Genebank

Knowledge of existing crop diversity, its distribution and evolution over time, is an essential pre-requisite for developing and implementing effective and efficient management strategies of crops and their genetic diversity. One of the mandates of genebanks is to assess and monitor genetic erosion and arrest the situation. The centre has recorded little achievement in the area of genetic erosion assessment.

Recent Developments

Recently (2021), the centre being the focal point for PGRs conservation and management in Nigeria with FAO prepared the country report on the state of PGRFA in preparation for the third report on the state of PGRFA in the world. The centre has instituted the National Committee on PGRFA to strengthen the linkages among different stakeholders as part of the implementation of the second world report.

Nigeria recently approved Nigeria's ratification of Plant Treaty in January, 2021. This ratification would change the status of the country from observer to full member of the treaty.

Moreover, the Centre organised a National summit and conference on Genetic Resources Conservation and Utilization held on 23-24 March, 2021 with a representation of international organisations such as IITA, FAO, ICRISAT and NEXTGEN CASSAVA. The centre has initiated action to

implement the recommendations of this summit.

Conclusion

This paper discussed the current exploitation status of plant genetic resources conservation in NACGRAB, Nigeria. It briefly discussed on the activities such as collection, storage, utilization, research, collaboration and management. The paper also identifies gaps in the area of genetic erosion assessment, limited funding, lack of synergy and network among organization managing Plant Genetic Resources.

In future, NACGRAB has to improve on the monitoring of Genetic erosion. In addition, there should be a strong communication network established among public, private, community based organizations, NGOs and international organizations for PGRs management. There should be awareness about the importance of plant genetic resources conservation among farmers and local people to establish community genebank and Link to National Genebank for proper plant genetic resources management.

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RESEARCH ARTICLE

Effect of *Fusarium oxysporum* f. sp. *lentis* on Seed Quality Parameters in Lentil

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Abstract

The Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lentis* (Fol) is recognized as a major seed and soil-borne disease of lentils worldwide. This disease not only affects yield but also cause deterioration of seed quality. The current study was carried out to determine the effect of Fol on seed quality parameters in lentils. Out of 120 samples collected from different agro-climatic regions of India, only 40 samples were used for seed quality parameter studies. The seed health testing method revealed the infection percentage of Fol-infected seed samples from 40 to 55%. We observed a significant reduction in the germination percentage (48.78%, Fol-102), seedling length (16.70%, Fol-125), and seedling vigour (882, Fol-135). In contrast, an increase in electrical conductivity (EC) (93.14 $\mu\text{S cm}^{-1} \text{ g}^{-1}$) was observed in Fol-119 infected seeds as compared to healthy seeds. Our results showed that the seed quality is highly compromised in Fol-infected seeds, compared to healthy seeds and needs to be addressed through the development of different management practices.

Keywords: Electric conductivity, Fusarium wilt, Germination, Lentil, Seed borne disease, Seedling length, Seedling vigour.

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Introduction

Lentil (*Lens culinaris* Medik.), commonly known as Masur, is an important pulse crop, mostly grown in the northern plains, central and eastern parts of India. The major lentil producing states are Madhya Pradesh, Uttar Pradesh, Bihar, Uttarakhand and Bengal. The main constraints responsible for the low yield of lentils include lack of availability of good quality seeds, biotic and abiotic stresses etc., Among the biotic stresses, several fungal, bacterial and viral diseases affect the crop's quality and yield. The potential threats for lentil cultivation are wilt (*Fusarium oxysporum* f. sp. *lentis*), collar rot (*Sclerotium rolfsii*), root rot (*Rhizoctonia solani*), rust (*Uromyces fabae*), powdery mildew (*Erysiphe polygoni*) and downy mildew (*Peronospora lentis*) (Javeria et al., 2020).

Among diseases, *Fusarium* wilt is the most widespread and important disease of lentil crop. Several species of *Fusarium* incite the disease but the most predominant fungus is *F. oxysporum* Schlecht. sp. *lentis* (Hiremani et al., 2016). It is one of the serious fungal diseases that cause maximum seed quality and yield loss in the crop by reducing crop stand in the field (Stoilova et al., 2006). Lentil diseases affect yield and cause deterioration of seed quality. The disease is estimated to cause economic yield losses in parts of South America, WANA, region, sub-Saharan Africa and South Asia. In Western Algeria, lentil was severely affected due to wilt (Tiware et al., 2018). The disease is both seed and soil borne and affects the seed germination, vigor to a great extent and attacks the crop both at the seedling and adult stages (Khare, 1996). In some fields, Fol

infection ranged from 25 to 95% and as high as 50 to 78% of wilt incidence has been reported in Madhya Pradesh (Agrawal *et al.*, 1991).

In India Fol is the main problem in states of Bihar, Madhya Pradesh, Uttar Pradesh, West Bengal and other areas where lentil is grown (Saxena *et al.* 2018). A survey of 116 districts in nine lentil-growing states of India recorded a plant mortality range of 0.7 to 9.3% at the reproductive stage. A loss of 37.5% grain yield per hectare and reduced marketability due to the discoloration of seed (Chaudhary *et al.*, 2012). The seed-borne pathogens and the mortality caused by them in major lentil growing areas of India during flowering to pod maturity are more crucial from a yield point of view and for carryover of the pathogens through seed (Chaudhary *et al.*, 2012). To increase the production of lentil qualitatively and quantitatively farmers requires quality seeds, with a high percentage of germination and purity. Hence, seeds must be tested before they are sown in the field. Another adverse effect of seed-borne pathogens is that they will contaminate areas that previously were disease-free. In this context, present studies were undertaken to determine the effect of Fol on seed quality parameters in lentils.

Materials and Methods

Survey and Sample Collection of Fol Infected Seed

The present study was conducted in different geographical regions of India i.e. Madhya Pradesh, Rajasthan, Bihar, Uttar Pradesh, Jharkhand, New Delhi and Chandigarh during *rabi* season in the year of 2018-2019 (Table 1 and Figure 1). More than 120 disease samples (Infected plant and infected seeds) of lentil wilt were collected at podding and harvesting stage. Wilted lentil plants and seeds were identified based on their typical wilt symptoms like drooping, epinasty, yellowing, xylem necrosis, discoloration and shriveled seeds. The wilt incidence was recorded (Figure 2).

Seed Quality Analysis

The wilt-infected seeds were surface sterilized with sodium hypochlorite 1% for 1 to 2 minute. Sterilized seeds were placed on PDA and incubated for 7 days at $22 \pm 1^\circ\text{C}$ with a 12 hours alternate cycle of light and dark in BOD. Both healthy and infected seeds were subjected to seed health, standard germination test and seed vigor were evaluated by using ISTA rules, 2020.

Seed Health Testing

To know the efficacy of seed health testing methods i.e. 2,4-D blotter and agar plate methods, were used and evaluate the best seed health testing method. The infection percent of Fol-infected seed samples were recorded.

2,4-D Blotter Method

Total 400 infected seeds of lentil were tested by employing 2,4-D blotter method in 4 replications. Three pieces of

blotting paper of 90 mm size were moistened with 0.2% solution of the sodium salt of 2, 4-dichlorophenoxy acetic acid and placed in 90 mm sterilized petri plates. seeds were placed at the rate of 25 seeds per petri plate at an

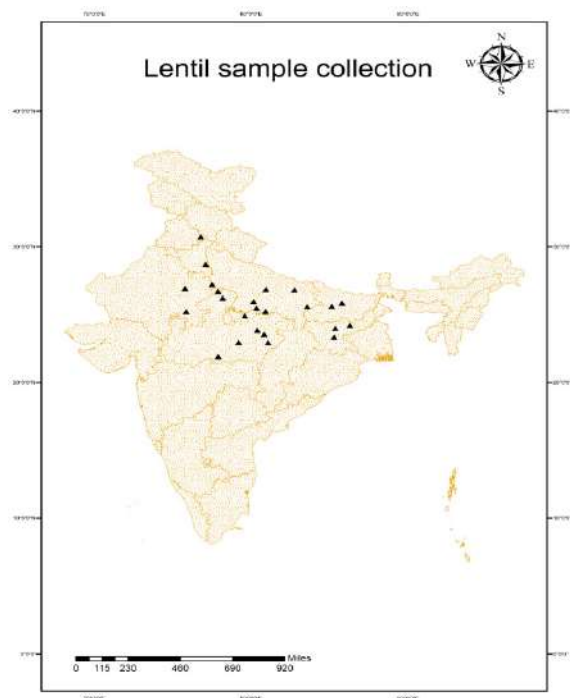


Figure 1: Map of India showing different states of collection of *Fusarium oxysporum* f. sp. *lentis* samples.

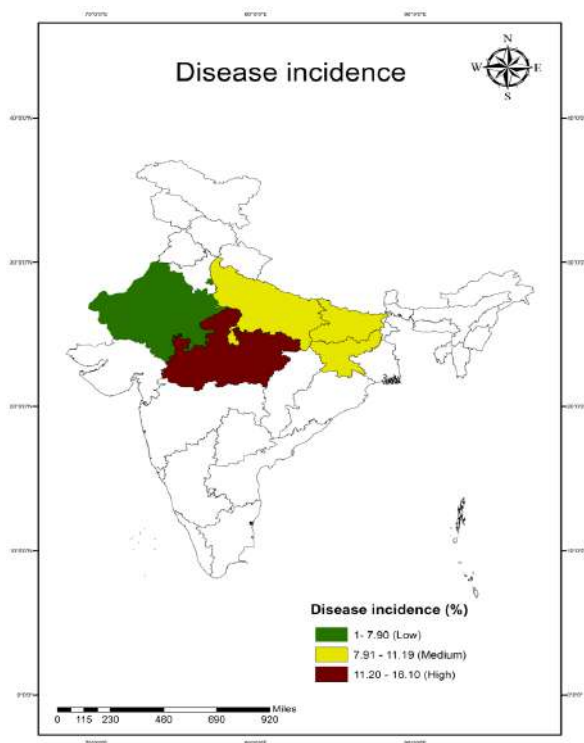


Figure 2: The disease incidence of lentil wilt was demarcated with different colors in these states.

Table 1: List of different *F. oxysporum* f. sp. *lentis* sample collected from the different geographical location.

S. No. and state	Isolate Name	District	Place
<i>Madhya Pradesh</i>			
1	Fol-101	Sehore	Sehore
2	Fol-102	Chatarpur	Gulganj
3	Fol 103	Gwalior	Badnapur
4	Fol -104	Umariya	Chandiya
5	Fol-105	Betul	Sonman
6	Fol-106	Katani	Kataria
7	Fol-107	Narsingpur	Tendukheda
8	Fol-108	Dindori	Shahpur
<i>Rajasthan</i>			
9	Fol-109	Bharatpur	Nimayapur
10	Fol-110	Bharatpur	Lulhara
11	Fol-111	Kota	Dabarha
12	Fol-112	Dholapur	Sevapur
13	Fol-113	Bharatpur	Nimayapur
14	Fol-114	Jaipur	Durgapur
<i>Bihar</i>			
15	Fol 115	Patna PPPP	PCR Mokama
16	Fol-116	Patna	PCR Mokama
17	Fol-117	Samastipur	TCA Pusa
18	Fol-118	Samastipur	TCA Pusa
19	Fol-119	Bhagalpur	Rajpur
20	Fol-120	Bhagalapur	Sabour
<i>Uttar Pradesh</i>			
21	Fol-121	Mhoba	Chokasora
22	Fol-122	Band	Gureh ka purva
23	Fol-123	Band	Badusa
24	Fol-124	Karvi	Pahari
25	Fol-125	Hamirpur	Muskara
26	Fol-126	Lucknow	Rahmatnagar
27	Fol-127	Gazipur	Shadibadi
28	Fol-128	Basti	Saoonghat
<i>Jharkhand</i>			
29	Fol-129	Hazaribagh	Hazaribagh
30	Fol-130	Ranchi	PL-156
31	Fol-131	Giridih	Mehgaon
32	Fol-132	Chatra	Intaroti
<i>Delhi</i>			
33	Fol-133	Delhi	IARI field
34	Fol-134	Delhi	IARI field
35	Fol-135	Delhi	IARI field
36	Fol-136	Delhi	IARI field
<i>Chandigarh</i>			
37	Fol-137	Chandigarh	Local field
38	Fol-138	Chandigarh	Local field
39	Fol-139	Chandigarh	Local field
40	Fol-140	Chandigarh	Local field

equal distance. The petri plates were incubated at room temperature ($22 \pm 1^\circ\text{C}$) under alternate cycles of 12 hours NUV light and darkness. After 7 days of incubation, the fungal growth was examined under a stereoscopic binocular microscope (40X) (ISTA rules 2020).

Agar Plate Method

For 400 seeds of lentil wilt infected seeds were surface sterilized with 1% sodium hypochlorite solution for 1 to 2 min and then placed at the rate of 10 seeds per petri plate containing 20 mL of potato dextrose agar (PDA). The petri plates were incubated at room temperature ($22 \pm 1^\circ\text{C}$) under alternate cycles of 12 hours NUV light and darkness. After 7 days of incubation, the fungal growth was examined under a stereoscopic binocular microscope (40X) (ISTA rules 2020).

Standard Germination Test

Total 400 seeds were used for conducting a standard germination test. With the help of a counting board, hundred seeds for each replicate were placed between two moist germination papers. Then the germination papers were folded and rolled up carefully along one edge, ensuring no excess pressure was placed on the seeds. These were wrapped in wax paper to prevent surface evaporation and placed in an upright position in a germinator at $25 \pm 1^\circ\text{C}$ and 90% relative humidity. After 5 days of incubation, the seedlings were examined for normal, abnormal seedlings, fresh un-germinated and dead seeds following the International Rules for Seed Testing (2020). A second count of abnormal seedlings was made after the completion of 14 days. Seedling length and seed dry weights were recorded and seedling vigor index I and II were calculated, respectively as suggested by (Abdul Baki and Anderson, 1973).

Electrical Conductivity

Four subsamples of 50 seeds were weighed (0.001 g accuracy) and put in plastic cups containing 75 mL deionized water in a BOD-type germination chamber at 25°C for 24 hours. The electrical conductivity (EC) of soaking solution was measured after this period, and the findings were represented in $\mu\text{Scm}^{-1}\text{g}^{-1}$ of seed (AOSA, 1983).

Statistical Analysis

The data recorded were subjected to statistical analysis by adopting a complete block design using OPSTAT and the percentage data were transformed into arcsine value for analysis.

Results and Discussion

In the present study, a survey of lentil growing areas of Madhya Pradesh, Rajasthan, Bihar, Uttar Pradesh, Jharkhand, New Delhi and Chandigarh states lentil wilt indicated that the wilt incidence varied in different areas (Figure 2). Average of 16.2% wilt incidence was found in Madhya Pradesh

Table 2: Effect of *F. oxysporum* f. sp. *lentis* on seed quality parameters of lentil.

S No.	Isolate Name	Germination (%)		Seedling length (cm)		Seedling vigor		Electrical conductivity ($\mu\text{S cm}^{-1} \text{g}^{-1}$)	
		HS	IS	HS	IS	HS	IS	HS	IS
1	Fol-101	82.34	67.32	26.22	18.34	2159	1235	73.56	88.78
2	Fol-102	78.23	48.78	28.04	21.54	2194	1051	64.87	87.57
3	Fol-103	79.45	59.77	27.46	19.56	2182	1169	75.18	89.39
4	Fol-104	81.44	69.32	23.34	20.33	1901	1409	64.86	82.67
5	Fol-105	80.21	58.34	21.43	17.34	1719	1012	59.35	72.13
6	Fol-106	77.34	63.50	27.44	19.68	2122	1250	78.96	88.08
7	Fol-107	85.42	68.21	24.32	22.39	2078	1527	61.55	76.14
8	Fol-108	83.32	59.33	25.20	19.56	2100	1161	50.73	67.61
9	Fol-109	79.85	70.22	27.97	17.44	2234	1226	67.02	81.11
10	Fol-110	76.12	61.50	24.20	18.40	1842	1133	61.37	79.51
11	Fol-111	79.80	68.78	25.12	21.97	2005	1511	68.42	81.05
12	Fol-112	81.34	63.22	25.47	20.40	2072	1290	63.64	83.94
13	Fol-113	76.45	58.45	23.26	20.56	1779	1202	41.71	62.31
14	Fol-114	76.45	56.66	27.79	21.65	2125	1227	53.86	81.69
15	Fol-115	76.45	53.21	29.34	19.80	2243	1054	50.11	68.86
16	Fol-116	76.45	63.22	27.47	18.12	2101	1146	60.80	71.43
17	Fol-117	78.72	57.33	27.89	19.45	2196	1115	80.69	91.99
18	Fol-118	83.32	69.32	24.35	20.85	2029	1446	71.66	81.87
19	Fol-119	81.12	58.67	29.22	19.34	2371	1135	88.55	93.14
20	Fol-120	82.78	69.80	21.45	20.85	1776	1456	87.58	91.54
21	Fol-121	77.23	61.32	26.43	18.23	2041	1118	89.28	91.54
22	Fol-122	79.52	68.34	25.08	16.75	1995	1145	59.06	72.16
23	Fol-123	81.76	67.53	24.04	18.90	1966	1277	70.24	80.73
24	Fol-124	79.80	56.45	22.78	17.56	1818	991	63.50	80.87
25	Fol-125	82.43	58.45	24.48	16.70	2018	976	57.63	69.28
26	Fol-126	82.23	71.43	23.80	22.67	1958	1619	69.97	81.67
27	Fol-127	78.89	68.23	27.83	17.54	2196	1197	52.07	68.22
28	Fol-128	75.75	73.23	29.89	21.78	2265	1595	52.96	73.72
29	Fol-129	89.76	63.22	23.20	18.32	2082	1158	50.42	71.14
30	Fol-130	83.21	54.32	26.23	19.00	2183	1032	53.54	74.31
31	Fol-131	87.43	65.35	22.86	17.54	1999	1146	64.75	78.09
32	Fol-132	79.01	62.24	28.57	19.04	2257	1185	67.79	77.07
33	Fol-133	86.87	57.43	23.56	18.23	2047	1047	79.19	91.86
34	Fol-134	83.45	54.42	23.56	16.20	1966	882	78.61	89.51
35	Fol-135	78.41	65.78	27.34	19.01	2144	1250	62.37	71.23
36	Fol-136	79.40	66.20	26.19	18.34	2080	1214	56.55	75.52
37	Fol-137	86.21	70.78	28.37	16.00	2446	1132	67.42	85.35
38	Fol-138	82.33	54.32	26.51	18.09	2183	983	69.56	87.39
39	Fol-139	82.32	52.43	23.16	22.00	1907	1153	75.51	91.04
40	Fol-140	78.85	52.91	27.83	21.61	2195	1143	69.92	82.30
	C.D.	3.566	3.928	1.133	1.472	97.782	51.104	1.965	2.395
	SE (m)	1.265	1.393	0.402	0.522	34.678	18.124	0.697	0.85
	C.V.	2.712	3.877	2.706	4.69	2.896	2.616	1.92	1.831

followed by the moderate incidence of wilt 7.09, 9.23, 11.19 and 9.99% in Rajasthan, Bihar, Uttar Pradesh and Jharkhand, while 4.8% average wilt incidence was noticed in Delhi. Chaudhary *et al.* (2010) have reported average mortality 6.3% due to soil-borne pathogens where in, *F. oxysporum* f.sp. *lentis* was associated in 62% cases. *F. oxysporum* f.sp. *lentis* has also remained the most crucial limiting variable for decreasing yield levels of lentil (Parihar *et al.* 2017).

Among the 120 of samples, 40 samples were selected for further study. Out of the 40 samples, 8 were from Madhya Pradesh, 8 from Uttar Pradesh, 4 from Jharkhand, 6 each from Bihar and Rajasthan and four from Delhi and Chandigarh were used for analysis of seed quality parameters (Table 1). The seed health testing was conducted for both infected and healthy seed samples by using 2-4 D blotter method and Agar plate method (Figure 3). The results of this study indicate that more than 52.55% (Fol- MP (102) infection was seen in 2-4 D blotter method as compared to agar plate method (33.11%). The lowest infection per cent was 29.18% in 2-4 D method but in case of agar plate method lowest infection percent was (19.19%) (Figure 4). It indicates that the 2, 4- D blotter method is an effective method for the detection of Fol fungi. The moistening of blotter paper with 0.2% 2,4-D solution, which enhances the growth of slow-growing seed pathogen, suppress the germination of seed and suppress saprophytic fungi which impair the detection of pathogenic fungi on seed (Naimuddin and Chaudhary.

2-4-D Blotter method

*Fusarium oxysporum* f.sp. *lentis*

Agar plate method

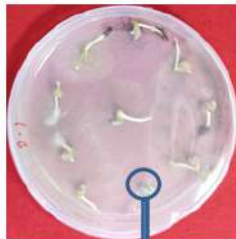
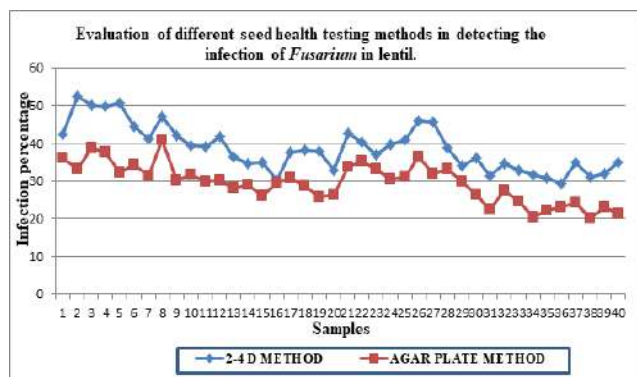
*Fusarium oxysporum* f.sp. *lentis*

Figure 3: Seed health testing method



Supplementary Figure 1: Evaluation of different seed health testing methods in detecting the infection of *Fusarium* in lentil.

2009; Singh *et al.*, 2010; Dawar *et al.*, 2007). The finding of the present study was in concurrence with Mohamed *et al.* (2011), where they find that 2,4-D blotter method is an effective method for the detection of some important seed-borne pathogenic fungi (*Cephalosporium* sp., *Fusarium verticilloides*, *F. oxysporum*, *F. solani* and *Verticillium* spp.) in peanut seeds. Hence 2,4-D blotter method is most effective for the detection of infection of seed borne *Fusarium* spp.

No published information is available from our country regarding seed quality deterioration studies in Fol. The effect of Fol on seed quality parameters like germination, seedling length, dry seedling weight, seedling vigor and EC were recorded. The present study revealed the infected seeds significantly reduced the seed quality parameters compared to healthy seeds (Table 2). Based on the experimentation, the germination percentage of the infected seed sample ranged from 48.78 to 70.78% and minimum germination was recorded in the sample Fol-102 (48.78%) which was collected from Madhya Pradesh. In case of a healthy seed lot germination percentage ranged from 75.75 to 89.76% (Figure 5a). It indicates that the 30% reduction was observed in infected seed samples as compared to healthy seed samples (Figure 5b). Similarly, the reduction in the seedling length was observed in infected seed samples as compared to healthy seed. The seedling length of infected seed samples ranges from 16.00 to 22.67 cm and minimum

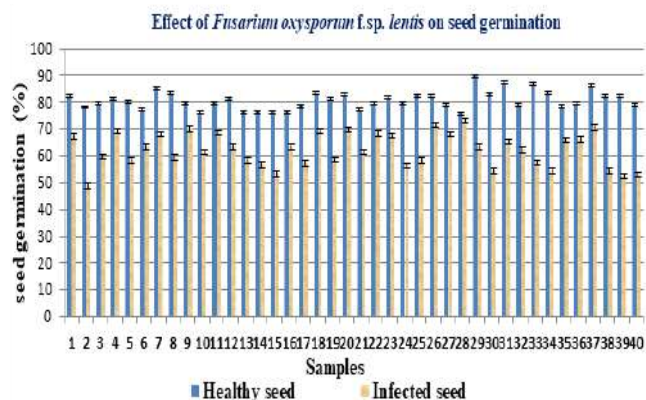


Figure 5 (a): Effect of *Fusarium oxysporum* f.sp. *lentis* on seed germination

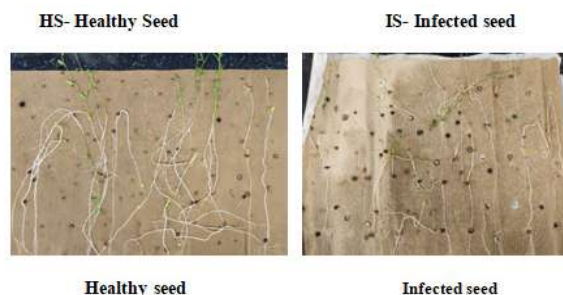


Figure 5 (b): Effect of *Fusarium oxysporum* f.sp. *lentis* on seed germination.

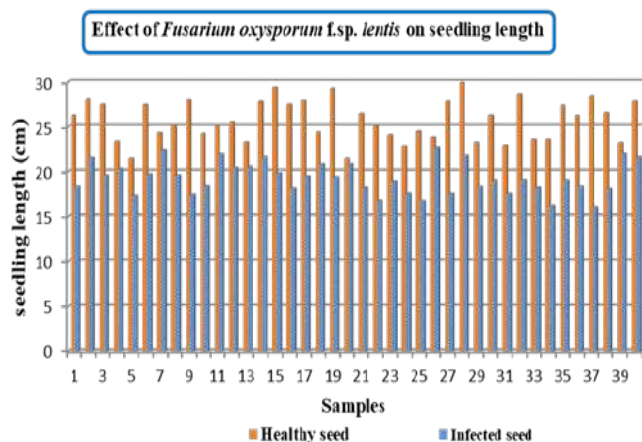


Figure 6a: Effect of *Fusarium oxysporum* f.sp. lentis on seedling length

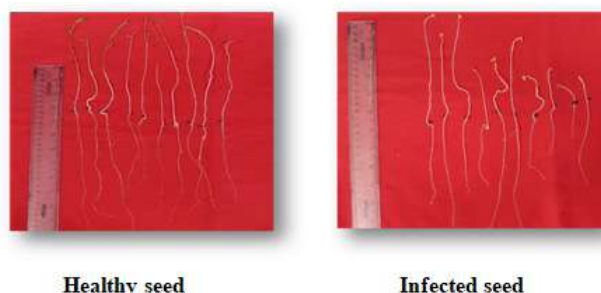


Figure 6b: Effect of *Fusarium oxysporum* f.sp. lentis on seedling length

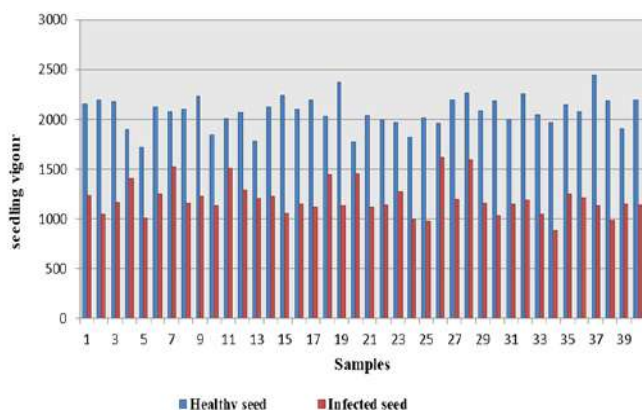


Figure 7a: Effect of *Fusarium oxysporum* f. sp. lentis on seedling vigor



Figure 7b: Effect of *Fusarium oxysporum* f. sp. lentis on seedling vigor

seedling length was observed in sample Fol-122 (16.75 cm) which was collected from Uttar Pradesh (Figure 6a). The highest seedling length was observed in healthy seed samples and ranges from 21.43 to 29.34 cm (Figure 6b). The minimum seedling vigor was observed in infected seed samples, it ranges from 882 to 1619 (Figure 7a). In case of healthy seed, seedling vigor ranges from 1719 to 2446. The highest seedling vigor was observed in Delhi isolate Fol-137 (2446) (Figure 7b). The highest EC was observed in the infected seed sample, it ranges from 62.31 to 93.14 $\mu\text{S cm}^{-1} \text{g}^{-1}$. The lowest EC was recorded in healthy seed samples it ranging from 41.71 to 89.28 $\mu\text{S cm}^{-1} \text{g}^{-1}$. The result of our study indicated that healthy seeds performed better, as compared to infected seeds in terms of seed quality parameters. The reduced seed quality parameters in infected seeds are due to shriveled seed, seed rotting, and seed infection. It makes the seeds vulnerable to seed deterioration it, ultimately causes reduced germination and poor crop establishment. Same variation of results was observed by Sadhu *et al.* (2014) who reported the *Fusarium moniliforme* drastically reduces seed germination (70%), root length (3.8 cm), shoot length (3.0 cm) and seedling emergence (70%) in green gram. Seed associated with *A. niger*, *A. tenuis*, *Penicillium spp*, *Cladopsorium spp.* and *F. moniliforme*, reduced the seedling length, seed germination and seed vigor drastically in pigeon pea also reported by Prasanna Kumar (2004).

Conclusion

Lentil wilt had a significant ($p < 0.05$) effect on seed quality parameters. The germination percentage, seedling length, and seedling vigor index I and II, reduces drastically while EC was increased in Fol-infected seeds. The noteworthy information produced would be helpful in analyzing the effect of Fol on seed quality parameters of lentil.

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RESEARCH ARTICLE

Assessment of Phenotypic Variations and Correlation among Yield Attributing and Biochemical Traits in Mutant Population of Grasspea (*Lathyrus sativus* L.)

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Abstract

The experiment was conducted during the winter season 2014-17 in West Bengal to assess the agro-morphological variation and to carry out association analysis among yield related traits, including biochemical parameters in the gamma rays and EMS derived a total of 51 mutant lines of grasspea. A wide range of variation among the mutant line was observed for 11 agronomic and maturity characters, including yield and three nutritional quality parameters (β -ODAP, protein and TIA). Significant reduction in β -ODAP content as compared to their respective parent was recorded in 5 mutant lines of Nirmal (0.293% β -ODAP), 6 lines selected from Biol-212 (0.107% β -ODAP) and 7 lines isolated from Berhampur Local (0.533% β -ODAP). An association study revealed that seed yield/ plant exhibited positive and significant ($p < 0.01$) correlation with plant height, primary branches/plant, secondary branches/plant, number of pods/plant, seeds/plant, 100 seed weight, fresh weight/ plant and seed protein content while, it showed highly negative correlation with anti-nutritional parameters like β -ODAP (-0.374) and TIA (-0.395). Meanwhile, the number of seeds/pod, days to 50% flowering and days to maturity did not exhibited any significant association with plant yield. On the contrary, maturity traits showed a strong positive association with β -ODAP and TIA and a strong negative correlation with seed protein content, which stipulates that a high yielding and early flowering mutants might have low β -ODAP content, which leads to the simultaneous improvement of yield, β -ODAP and protein content of the grasspea mutants under study.

Keywords: Biochemical parameters, Correlation, Grasspea, Mutant, Variation.

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Introduction

Grasspea is an important crop of economic significance in India, China, Bangladesh, Pakistan, Nepal and Ethiopia. India is the leading country of grasspea cultivation in area and production, ranking second in productivity after Bangladesh. Currently, in India, about 3.62 lakh ha area of land is under grasspea cultivation (Department of Agriculture and Farmers Welfare, 2021). It is one of the important pulse crop of West Bengal cultivated in Murshidabad, East Midnapur, South 24 Pargonas, Nadia, Malda, Coochbehar and Jalpaiguri districts etc. The area, production and productivity of grasspea in West Bengal during the period 2011-12 is 33.5 thousand hectares, 26.93 thousand tons and 804 kg/ha, respectively.

Grasspea is an underutilized and neglected food, feed and a pharmaceutically important versatile crop shows resistance to harsh environmental conditions (Kumar *et al.*, 2020 and Tripathi *et al.*, 2021). The crop known for its several unique properties like tolerance to various abiotic stress, very hardy and penetrating root system, low fertilizer and water responsiveness, crop residue acting as source of good green manure, grain sturdiness, and a good source of protein which make this otherwise neglected crop tolerant and adaptable to a wide range of agro-climatic conditions.

On contrary, the presence of anti-nutritional substances (β -ODAP and Trypsin Inhibitor) in seeds and other plant parts render the crop unsuitable for human consumption leads to banned the crop production in some Indian states under the Prevention of Food Adulteration Act 1961 (Arora *et al.*, 1995). This has resulted in reduction of its cultivation areas from 1.3 million hectares to less than 850,000 ha in a decade (Kumar *et al.*, 2020). Presently, in South Asia, low β -ODAP varieties disseminating to the farmers in the grasspea growing regions reduced the incidence of neurolathyrism considerably (Ramya *et al.*, 2022). Hence, identifying genotypes with high yield, high protein content and low anti-nutritional factors are the prime focus of any grass pea breeders and researchers worldwide that could help in achieving long lasting and dependable solution in efforts to face the threat of lathyrism.

Inadequate genetic variation in grasspea due to the predominant presence of self-pollination behavior and inter specific incompatibility hinders its improvement through conventional breeding which might be outbreak through induced mutagenesis (Singh and Sadhukhan, 2019). Mutagenesis and mutation breeding can be a valuable supplement to conventional breeding methods that can be introduced deliberately by employing ionizing radiation (γ -rays) as well as chemical mutagens like EMS (Singh *et al.*, 2019) to create additional genetic variability in the existing gene pool. Furthermore, to complement genetically engineered crops mutation breeding offers the benefit of not introducing foreign DNA as a form of genetic diversity, preventing the restriction and utilization of mutant plants (Thapa *et al.*, 2016).

Various metrical attributes like yield, number of branches, number of pods and seeds, leaf area, fresh weight etc. and biochemical traits are very complex as polygenes govern it and are greatly influenced by environmental factors. Further, an increase in yield is not the direct action of mutagen alone rather it also depends on other yield attributing interrelated characters hence direct selection for yield as such can be misleading. Intrinsically assessing variability through induced mutagenesis among various mutants for morphological and biochemical characters is highly important. The correlation of a particular trait with others contributes to the indirect selection of potential genotypes, which is important to understand the performance of mutants in comparison to the existing parent genotypes for desirable traits (Kadam *et al.*, 2014). So, indirect selection can use character associations between yield components as the best guide for successful yield improvement. Singh *et al.* (2017) reported the association of various traits with seed yield in the mutagenic population of grasspea. Moreover, earlier finding concerning correlation study among yield and yield attributing characters including biochemical traits through induce mutagenesis, have also

been reported by several researchers in chickpea (Hassan *et al.*, 2005), black gram (Baisakh *et al.*, 2014), moong bean (Ahmad *et al.*, 2012), soybean (Singh and Singh, 1999) *etc.* However, limited information is available regarding this aspect in grasspea. Considering the above fact and to contribute towards genotypic improvement of grasspea, the present investigation was carried out to assess the agro-morphological variation and the mode of association for various yield attributing traits, including biochemical parameters in the putative mutants of grasspea.

Materials and Methods

The present investigation was conducted at District Seed Farm, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India during the winter season 2014-17. The experimental site comes under the Gangetic plains of West Bengal, India at latitude 22°58' N and longitude 88°32' E with an average altitude of 9.75 m above mean sea level (AMSL). The experimental material consisted of three diverse genotypes of grasspea viz. Nirmal (V_1) selected from germplasm, Biol-212 (V_2) a Somaclone of cv. Pusa-24 (Report of All India Coordinated Research Project on Mungbean, Urdbean, Lentil, Lathyrus, Rajmash, and Pea, 2009) and Berhampur Local (V_3) locally collected from Berhampur (W.B.). Dry and healthy seeds (2500 for each variety) were subjected to different treatments of physical (400, 500 and 600 Gy gamma rays) and chemical (0.5 and 1% ethyl methane sulphonate) mutagens individually and in the combination of both (400 Gy of gamma rays followed by 0.5 and 1% EMS concentrations). So, a total of eight treatment combinations, including control (untreated seeds) were developed in each variety to raise the mutagenic population.

The mutagen-treated seeds were sown immediately in the main field along with their respective controls in a randomized block design (RBD) with three replications (total 300 seeds of each treatment) to raise the M_1 generation during 2014-15. In M_2 generation, a total of 900 seeds (in RBD 300 seeds for each treatment in 3 replication and 600 seeds in simple line method for each treatment) were grown during 2015-16, to maximize the mutagenic population for the selection of desirable mutants. Viable mutations were scored based on various morphological changes like plant habit, leaf morphology, flower color, seed morphology and color and maturity. Collectively from three varieties of grasspea, a total of 51 mutant families were isolated in M_2 generation (Table 1). These mutant families isolated from various mutagenic treatment comprising a different concentration of EMS (9 from 0.5% and 6 from 1% treatment), various irradiation doses of gamma rays (9 from 400Gy, 7 from 500Gy and 8 from 600Gy) and combination treatment of physical and chemical mutagens (4 from 400 Gy+0.5% EMS and 8 from 400 Gy+ 1% EMS).

All the 51 mutant lines selected in M_2 were evaluated in M_3 generation (2016-17) in RBD with three replications and

Table 1: List of viable mutants lines of grasspea selected from different mutagenic doses and their characteristic features

S. No.	Mutant line	Mutant type	Treatment detail
Parent: Nirmal			
1.	Nm1	High yielding	600 Gy
2.	Nm2	Flower color	400 Gy
3.	Nm3	Flower and leaf	400 Gy+1% EMS
4.	Nm4	Flower and seed	400 Gy
5.	Nm5	Flower color	1% EMS
6.	Nm6	Plant habit and seed color	400 Gy+1% EMS
7.	Nm7	Early maturity	1% EMS
8.	Nm8	Seed size	400 Gy+1% EMS
9.	Nm9	Seed size	0.5% EMS
10.	Nm10	Early maturity	1% EMS
11.	Nm11	Early maturity	1% EMS
12.	Nm12	Plant height	0.5% EMS
Parent: Biol-212			
13.	Biom1	High yielding	400 Gy+0.5% EMS
14.	Biom2	Chlorophyll	600 Gy
15.	Biom3	Plant habit	600 Gy
16.	Biom4	Chlorophyll with late maturity	0.5% EMS
17.	Biom5	Seed size with early maturity	0.5% EMS
18.	Biom6	Leaf shape	500 Gy
19.	Biom7	High yielding	400 Gy+ 1% EMS
20.	Biom8	Leaf size	500 Gy
21.	Biom9	Plant habit	400 Gy+ 1% EMS
22.	Biom10	Plant habit and early maturity	400 Gy+ 1% EMS
23.	Biom11	Plant habit	400 Gy
24.	Biom12	Flower color	400 Gy
25.	Biom13	High yielding	0.5% EMS
26.	Biom14	Flower color	400 Gy
27.	Biom15	Seed size and color	400 Gy
28.	Biom16	Plant habit and seed size	400 Gy
29.	Biom17	Seed size and Early maturity	400 Gy
30.	Biom18	Plant habit	500 Gy
31.	Biom19	Seed color	600 Gy
32.	Biom20	Seed size	500 Gy
33.	Biom21	Seed color	500 Gy
34.	Biom22	Seed size	600 Gy
35.	Biom23	Seed size	1% EMS
36.	Biom24	Plant habit	0.5% EMS
37.	Biom25	Plant height	0.5% EMS

38.	Biom26	Leaf shape	600 Gy
39.	Biom27	Leaf shape	400 Gy+ 1% EMS
40.	Biom28	Leaf shape	400 Gy+0.5% EMS
41.	Biom29	Seed color	500 Gy

Parent: Berhampur Local			
42.	Blm1	High yielding	400 Gy+0.5% EMS
43.	Blm2	Seed size	0.5% EMS
44.	Blm3	High yielding	400 Gy+0.5% EMS
45.	Blm4	Seed color	600 Gy
46.	Blm5	Chlorophyll with high yielding	0.5% EMS
47.	Blm6	Flower color and plant habit	1% EMS
48.	Blm7	Seed size	500 Gy
49.	Blm8	Seed size and color	400 Gy+1% EMS
50.	Blm9	Plant height	400 Gy
51.	Blm10	Leaf shape	600 Gy

"Nm" denotes the Nirmal mutant, "Biom" denotes the Biol-212 mutant "Blm" denotes the Berhampur local mutant.

yield attributing data and quality parameters were recorded. Eleven agronomic and maturity characters namely plant height (cm), number of primary branches/ plant, number of secondary branches/plants, number of pods/plant, number of seeds/pod, number of seeds/plant, 100 seed weight (g), fresh weight/plant (g), days to 50% flowering, days to physiological maturity and seed yield/plant (g) along with three nutritional quality parameters (β -ODAP, protein and TIA) were accessed in selected mutant lines.

The nutritional parameters were only estimated from the seed (powder) part. The protein content, using bovine serum albumin (BSA) as a standard protein was analyzed using Lowry's method (Lowry *et al.*, 1951). The trypsin inhibitor activity is measured indirectly by inhibiting the activity of trypsin. The subsequent analysis of trypsin inhibitor activity was done following the method of Kakade *et al.* (1974) using N- α -benzoate-DL-arginine-paranitroanilide (BAPNA) hydrochloride as substrate solution. Estimation of β -N-oxalylamino-L-alanine (BOAA) is also known as β -N-oxalyl-L- α - β -diamino propionic acid (β -ODAP) in *Lathyrus* seeds was assessed as per the method given by Rao (1978).

Five random plants of all the putative mutants were tagged in each replication to record 11 quantitative traits and estimate 3 biochemical parameters and mean data used for statistical analysis. The mean value of all the characters that showed significant variations were used to determine the simple correlation coefficients according to Khan and Khanum (1994).

Results and Discussion

Variability studies among the selected mutant showed highly significant differences for all the yield attributing and quality traits except for number of days required to physiological

maturity in mutant lines of variety Berhampur Local (Table 2). This indicates the presence of considerable variability among the selected mutants of grasspea. The chronicled mean values for different agro-morphological and nutritional parameters have been displayed comparatively (Figure 1). A subset of 29, 12 and 10 M₃ lines from the variety Biol-212, Nirmal and Berhampur local, respectively were assessed to measure the yield attributes and seed composition traits like protein, β -ODAP and trypsin inhibitor activity. The putative mutant lines showed a broad range of variability for these yield attributing, maturity and biochemical traits (Figure 2).

Wide range of variation among the mutant line was observed for the yield attributing traits like plant height (53.45 to 118.45 cm in V₁, 29.65 to 121.15 cm in V₂ and 46.95 to 91.45 cm in V₃), primary branches/plant (4.47 to 9.17 in V₁, 5.38 to 12.50 in V₂ and 3.95 to 8.77 in V₃), secondary branches/plant (8.67 to 17.85 in V₁, 10.38 to 26.67 in V₂ and 12.67 to 23.75 in V₃), fresh weight/plant (41.30 to 92.55 g in V₁, 47.75 to 105.45 g in V₂ and 38.15 to 82.75g in V₃), number of

seeds/pod (3.33 to 4.67 in V₁, 2.83 to 4.33 in V₂ and 3 to 4.47 in V₃), number of seeds/plant (289.85 to 411.92 in V₁, 212.92 to 533.57 in V₂ and 316.92 to 473.27 in V₃), 100 seed weight (6.85 to 9.05 g in V₁, 6.78 to 12.15 g in V₂ and 5.65 to 8.43 g in V₃) and yield/ plant (21.58 to 34.20 g in V₁, 18.47 to 47.25 g in V₂ and 20.09 to 31.65 g in V₃).

Among the mutant lines extensive variability was also noticed for maturity indices. Days to 50% flowering ranged from 58 to 75 days in V₁, 64 to 78 days in V₂ and 78 to 89 days in V₃ whereas days to physiological maturity varied from 107 to 128 days in V₁, 112 to 130 days in V₂ and 122 to 134 days in V₃. Similarly, β -ODAP content ranged from 0.2 to 0.34%, 0.08 to 0.14% and 0.16 to 0.52% in the mutant lines of variety V₁, V₂ and V₃ respectively. Protein content varied between 22.75 to 31.07% in V₁, 23.97 to 30.47% in V₂ and 20.52 to 26.20 in V₃ while, the trypsin inhibitor activity ranged from 15.15 to 21.25 TIU mg⁻¹ of dry matter in V₁, 14.16 to 17.88 TIU mg⁻¹ of dry matter in V₂ and 23.69 to 27.83 TIU mg⁻¹ of dry matter in V₃.

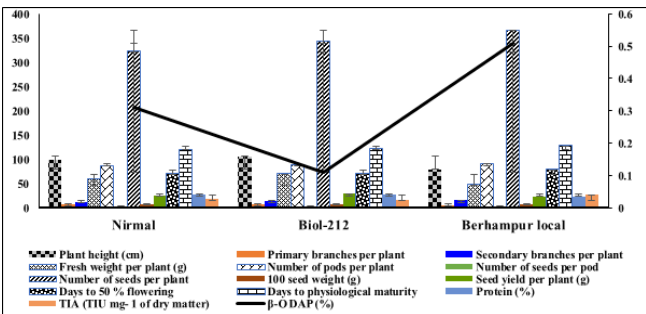


Figure 1: Mean performance of 51 putative mutants of three varieties for different yield attributing and biochemical traits in M₃ generation

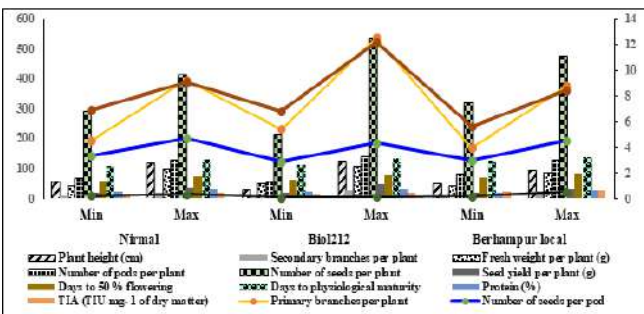


Figure 2: Range of 14 yield attributing and biochemical characters of 51 putative mutants selected from the variety Nirmal, Biol-212 and Berhampur Local

Table 2: Range value and mean performance for different yield attributing and biochemical traits in M₃ generation of 51 putative mutants isolated from three varieties

Characters	Nirmal (V ₁)			Biol212 (V ₂)			Berhampur Local (V ₃)		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Plant height (cm)	53.45	118.45	99.32	29.65	121.15	104.10	46.95	91.45	77.78
Primary branches per plant	4.47	9.17	6.25	5.38	12.50	7.31	3.95	8.77	4.52
Secondary branches per plant	8.67	17.85	11.19	10.38	26.67	13.99	12.67	23.75	15.52
Fresh weight per plant (g)	41.30	92.55	58.98	47.75	105.45	69.40	38.15	82.75	47.11
Number of pods per plant	67.73	125.81	85.93	54.87	138.63	89.24	77.67	124.33	91.22
Number of seeds per pod	3.33	4.67	3.69	2.83	4.33	3.72	3.0	4.47	3.71
Number of seeds per plant	289.85	411.92	322.17	212.92	533.57	343.39	316.92	473.27	365.28
100 seed weight (g)	6.85	9.05	7.42	6.78	12.15	7.86	5.65	8.43	6.16
Seed yield per plant (g)	21.58	34.20	25.24	18.15	47.25	28.54	20.09	31.65	23.33
Days to 50 % flowering	58.00	75.00	70.05	64.00	78.00	71.23	71.00	83.00	78.89
Days to physiological maturity	107.00	128.00	119.93	112.00	130.00	121.95	122.00	134.00	127.60
β -ODAP (%)	0.20	0.34	0.31	0.08	0.14	0.11	0.16	0.52	0.51
Protein (%)	22.75	31.07	28.16	23.97	30.47	27.47	20.52	26.20	23.51
TIA (TIU mg ⁻¹ of dry matter)	15.15	21.25	19.28	14.16	17.88	16.80	23.69	27.83	26.34

The mutational change in each quantitative trait can be large or small. Such changes in both macro and micro mutation are of significance in breeding programme depending on the characters involved. In the present investigation, isolated mutant lines exhibited variation in both positive and negative direction for all the traits under study. Meanwhile, in comparison to parents few lines showed significant reduction in mean value for maturity and anti-nutritional characters (β -ODAP and TIA) while few had substantial increase value of protein and other yield attributing traits over the control. Significant reduction in β -ODAP content as compared to their respective parent was recorded in *Nm5*, *Nm6*, *Nm7*, *Nm8*, *Nm11*, lines of Nirmal (0.293%), *Biom10*, *Biom17*, *Biom19*, *Biom20*, *Biom21* and *Biom22* lines selected from Biol-212 (0.107%) and *Blm1*, *Blm3*, *Blm4*, *Blm6*, *Blm7*, *Blm8* and *Blm9* lines isolated from Berhampur Local (0.533%). Isolation of such putative mutants will lead to the genetic improvement of grasspea and also enrich the germplasm. Various macro mutants were earlier reported in grasspea and other leguminous crops are in accordance with the present findings. Early maturity mutants have been reported by Bawankar and Patil (2001); Makeen *et al.* (2013) and Auti (2012) in grasspea, black gram and greengram, respectively. Similarly, plant habit mutants like dwarf (Talukdar, 2009a), reduction of lateral branches and biomass (Rybinski *et al.*, 2004) were isolated in grasspea. High yielding macro-mutants were earlier isolated by Tripathy and Lenka (2010) in grasspea, Makeen *et al.* (2009) in urdbean and Tripathy (2009) in mungbean are in agreement of few high yielding line isolated in present study. Biochemically significant (low β -ODAP, low TIA and high protein) mutant lines were also selected during present investigation are supported by similar findings reported earlier by Das and Kundagrami (2005) and Tripathy and Lenka (2010) in *Lathyrus* for low β -ODAP, Kaveri (2009) in groundnut, Yathaputanon *et al* (2009) in soybean and Kamble and Patil (2014) in chickpea for higher protein mutants.

Association analysis for all the characters was worked out in all possible combinations at phenotypic levels (Table 3).

Traits like plant height, primary and secondary branches, pods, seeds, 100 seed weight, fresh weight and seed protein content exhibited significant ($p < 0.01$) positive association with yield/plant. Seed β -ODAP content (-0.374) and TIA (-0.395) had highly negative correlation with yield. Positive association of seed yield with most of the yield attributing traits except seed β -ODAP content exhibited independent genetic behavior in grasspea (Pandey *et al.*, 2000). Sharma *et al.* (2000) reported significant negative correlation between seed yield/plant and days to flowering, days to maturity, pod length and its positive association with pods/plant in grasspea. Strong positive correlation of seed yield with protein content in grasspea was earlier reported by Basaran *et al.* (2016) which is in strong agreement of

present finding. Talukdar (2009) in grasspea recorded positive and statistically significant correlation between seed yield and plant height, number of primary branches, number of pods and 100 seeds weight.

In general, correlations between β -ODAP and other traits were either negative or nonsignificant. Conversely, β -ODAP exhibited strong positive association with TIA and significant positive correlation with days to 50% flowering and days to physiological maturity. Similarly, negative correlation of β -ODAP with seed yield, protein content and days to flowering were observed earlier by Basaran *et al.* (2016) and Talukdar (2009) in grasspea. Likewise, a negative correlation between β -ODAP and seed yield was reported earlier by Tadesse and Bekele (2003) and Basaran *et al.* (2013). However, Cocks *et al.* (2000) reported a negative relationship between ODAP concentration and the total amount of ODAP in the plant (total seed weight x ODAP concentration) and proposed that low toxin concentration in plant with high seed yielding were caused by toxin dilution. So, the negative and significant correlation between β -ODAP and yield should be taken into consideration for simultaneous improvement of these traits.

Seed protein content had highly significant positive correlation with plant height and 100 seed weight and positive significant association with Seed yield/plant. on contrary, showed highly negative and significant association with days to 50% flowering, days to physiological maturity and seed β -ODAP content. The above finding suggests that protein content and yield can be improved simultaneously. Similar findings have also been reported by Roy *et al.* (2001) in grasspea.

Trypsin Inhibitor Activity (TIA) showed highly significant positive correlation with days to 50% flowering, days to physiological maturity and β -ODAP. While, it exhibited strong negative relationship with most of the traits, including yield/plant. A negative correlation between TIA and seed yield and seed protein was earlier observed in field pea and grass pea (Wang *et al.*, 1998). Whereas, no correlation of TIA with maturity, oil content, seed protein content and seed yield was reported by Manjaya *et al.* (2007) in soybean. Strong positive relation between TIA and days to physiological maturity and highly negative association with seed yield in the present study indicates that a high yielding and early flowering mutants might also have low TIA content which may leads to concurrent improvement of seed yield and TIA in grasspea.

Plant height showed highly positive significant correlation with number of primary branches, number of pods, number of seeds plant, 100 seed weight, fresh weight, seed protein content and seed yield. While, negative and significant relationship recorded with number of seeds/pod and seed β -ODAP content and strong negative association with TIA. Basaran *et al.* (2013) reported that plant

Table 3: Simple correlation coefficient among different yield attributes protein, β -ODAP content and trypsin inhibitor activity (TIA) in control and 51 putative mutants of grasspea in M₃ generation

Characters	PH	PB	SB	PP	SP	SPP	SW	FWP	DFF	DPM	ODAP	SP	TIA
PB	0.561**												
SB	0.254	0.736**											
PP	0.489**	0.626**	0.779**										
SP	-0.285*	-0.249	-0.289*	-0.357**									
SPP	0.391**	0.545**	0.666**	0.843**	0.190								
SW	0.390**	0.230	0.070	0.043	-0.466**	-0.213							
FWP	0.674**	0.706**	0.543**	0.548**	-0.276*	0.436**	0.448**						
DFF	-0.086	0.081	0.448**	0.428**	-0.109	0.393**	-0.216	0.047					
DPM	-0.006	0.131	0.481**	0.461**	-0.143	0.410**	-0.120	0.157	0.934**				
ODAP	-0.318*	-0.274*	-0.065	0.083	0.044	0.076	-0.545**	-0.414**	0.276*	0.288*			
SP	0.412**	0.200	-0.210	-0.130	-0.037	-0.153	0.544**	0.177	-0.438**	-0.370*	-0.417**		
TIA	-0.485**	-0.296*	0.072	0.096	0.022	0.101	-0.577**	-0.443**	0.481**	0.441**	0.717**	-0.661**	
SYP	0.637**	0.626**	0.580**	0.709**	-0.201	0.636**	0.609**	0.716**	0.127	0.220	-0.374**	0.328*	-0.395**

*Significant at 5%; ** Significant at 1%

PH = Plant height (cm), PB = No. of primary branches, SB =No. of secondary branches, PP =No. of pods / plant, SP =No. of seeds / pod, SPP =No. of seeds / plant, SW= 100 seed weight (g), FWP= Fresh weight / plant (g), DFF= Days to 50% flowering, DPM= Days to physiological maturity, ODAP= Seed β -ODAP content (%), SP= Seed protein (%), TIA= Trypsin Inhibitor Activity (TIU mg-1 of dry matter), SYP= Seed yield / plant (g)

height exhibited strong correlation with seed yield and a nonsignificant positive and negative association with seed protein content and β -ODAP, respectively, which supports the present finding. Furthermore, a dwarf mutant line can be taken in consideration for the simultaneous enhancement of yield and quality parameters in *Lathyrus*. The number of primary branches plant had strong positive associations with secondary branches, number of pods, seed plant, fresh weight and seed yield whereas, it showed significant negative correlation with seed β -ODAP content and TIA. The number of secondary branches exhibited a highly positive correlation with plant height, number of pods, number of seeds plant, fresh weight, days to 50% flowering, days to physiological maturity and seed yield. However, it had significantly negative relationship with number of seeds pod.

Number of pods plant exhibited positive and highly significant correlation with plant height, number of primary and secondary branches, number of seeds, fresh weight, and days to 50% flowering, days to physiological maturity and seed yield plant while it had strong negative association with number of seeds pod. Similarly, seeds pod showed significant negative correlation with most of the traits but did not have significant positive association with any of the character. Strong positive association was recorded between number of seeds plant with plant height, primary and secondary branches, pods plant, fresh weight, days to 50% flowering, days to physiological maturity and seed yield plant whereas, there was no negative correlation observed with any other characters. 100 seed weight showed highly positive and significant correlations with plant height, fresh weight, seed protein content and seed yield/plant while,

it had strong negative association with number of seeds/pod, seed β -ODAP and TIA.

Fresh weight/plant exhibited strong positive correlation with plant height, number of primary and secondary branches, number of pods, seeds plant, 100 seed weight and seed yield while it showed highly negative association with β -ODAP and TIA. So, a bushy type mutant with high fresh weight might have low β -ODAP and TIA can be selected for fodder purposes and utilized in future breeding programs. Days to 50% flowering and days to physiological maturity were positively associated with number of secondary branches, pods plant, seeds plant, TIA and β -ODAP content. On other hand, it had highly negative correlation with seed protein and nonsignificant relation with seed yield. Kumar and Dubey (2001) reported significant negative correlation between days to flowering and yield which is in agreement of present finding.

Conclusion

Consequently, in the present study few mutant lines exhibited lower concentrations of anti-nutritional factors while others had high value of protein content and grain yield than their respective control parent. Isolation of such lines would be beneficial for genetic improvement of grasspea. Further, important yield contributing characters like plant height, number branches, number of pods, number of seeds, 100 seed weight, fresh weight and protein content exhibited strong positive and significant correlation with yield. Henceforth, based on character association selection of these traits will be helpful to improve the grain yield. On contrary, TIA and β -ODAP exhibited strong negative association with plant yield and a significant negative

correlation with most of the important yield attributing traits along with protein but they were very strongly and positively associated with each other and also, they had positive association with maturity traits. Therefore, the result obtained in the present study indicates that a high yielding, early flowering and early maturing mutants may also have low β -ODAP and TIA content, leading to the concurrent improvement of yield in consort with anti-nutritional and protein of the grasspea mutants under study.

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RESEARCH ARTICLE

Characterization and Evaluation of Mulberry Genetic Resources for the Identification of Promising Accessions

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Abstract

Mulberry is a dioecious species but, is usually cultivated as low bush or dwarf tree form by repeated pruning. Apart from its importance in silkworm rearing, it is also valued for its delicious fruits, medicinal properties in infusions, and ornamental shade tree. The conservation of mulberry genetic resources for future use and their subsequent usage in plant breeding are two critical areas of action in any genetic resource management programme. The current study used morphological, reproductive, anatomical, biochemical, propagation, growth, and yield variables to know the potential value of 22 mulberry accessions. Based on multiple trait analysis, the accessions MI-0946, MI-0945, MI-0953, MI-0948, MI-0935, MI-0948, MI-0936 and MI-0952 were identified as top performers for anatomy, propagation, biochemical, growth and yield parameters. These accessions may serve as potential parents for the future breeding program.

Keywords: Mulberry germplasm, Characterization, Evaluation, Conservation.

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Introduction

Mulberry is a highly adaptable plant with numerous uses, the most notable of which is as the sole food source for the monophagous silkworm *Bombyx mori*. Plant genetic resources play an important role in crop improvement (Khurana and Checker, 2011). Mulberry genetic resources are becoming more widely acknowledged as a critical component for sustainable silk production in the face of changing environmental conditions. The current scenario of the sericulture industry necessitates the development of new varieties that are suitable for various agro-climatic conditions. From a huge number of germplasm collections, suitable parent material must be selected (Sharma *et al.*, 2000). Several studies have highlighted the variability of mulberry germplasm (Thangavelu *et al.*, 2000; Tikader and Rao, 2002).

The key to understanding germplasm's potential and actual value is to characterize and evaluate it. This will facilitate the utilization of germplasm in crop improvement programme. Thus, characterization and evaluation is an important activity and maintaining the germplasm without losing its genetic integrity is also important in plant genetic resources management. The clonal repositories, field gene banks and herbal gardens are strategies for the maintenance of germplasm of vegetatively propagated species. Characterization and evaluation of germplasm is gaining importance all over the world due to the utilization of plant genetic resources in crop improvement programme. Such activities are also consistent with the Convention on Biological Diversity under which countries agreed to conserve, sustainable use and benefit sharing from plant genetic resources. Therefore, characterization and

evaluation of germplasm is essential for conservation and utilization of plant genetic resources (Sharma *et al.*, 2000).

But, the genetic improvement of mulberry depends on the availability of diverse germplasm. Selection of suitable genotypes from gene pool requires a thorough knowledge on availability and distribution of traits of economic importance for utilization in hybridization programmes (Tikader and Kamble, 2008). Realizing the need for enhanced use of mulberry germplasm resources for crop improvement programmes, Central Sericultural Germplasm Resources Centre (CSGRC) has taken up characterization and evaluation of entire germplasm collection of the centre. A total of 1317 mulberry accessions (Indigenous-1032 and Exotic-285) are being conserved in the form of ex situ field gene bank. This germplasm are systematically characterized in phased manner and recently sixth volume of catalogue with data pertaining to 52 additional mulberry accessions was published (Thriveni *et al.*, 2021). In order to know the potential value of the mulberry germplasm, the characterization and evaluation is prerequisite. Studies have shown that the identification of trait-specific potential genotype(s) from a broad genetic base and their subsequent utilization in targeted breeding work are desirable. However, reports on mulberry genetic resources evaluation through multivariate analysis is still very limited (Banerjee *et al.*, 2011).

In this context, the present study was conducted to know the performance of mulberry germplasm accessions for different parameters for effective utilization in future breeding program. The characterization includes morphology, anatomy, and reproductive parameters. The evaluation parameters comprise propagation, growth and yield and biochemical traits.

Materials and Methods

Stem cuttings of 22 mulberry accessions with 12 to 15 cm length and 1.0 to 1.5 cm diameter from 6 to 8 months mature shoots with 2 to 3 healthy buds are planted in nursery beds. The characterization and evaluation parameters are observed after 70 to 90 days of planting. The methodology for recording the characterization data is given in Table 1.

Methodology for Characterization

Morphology

- Branching nature

The branching nature of plants were recorded based on visual observations from 3 plants/accession after 90 days of pruning and grouped into erect, semi-erect, and spreading.

- Curve or straightness of branch

Curve or straightness of the branches were scored based on visual observations from 3 plants/accessions and grouped as curved, slightly curved and straight depending on plant habit.

- Color of young shoot

Young shoot color were recorded on visual observations from 3 plants/accession after 30 days of pruning and scored as brown, green and purple.

- Color of mature shoot

Mature shoot color on visual observations from 3 plants/accession from the mature shoot after 90 days of pruning and grouped into brown, green, greenish brown, greenish grey and purple brown.

- Phyllotaxy

Phyllotaxy is the mode of arrangement of leaves on the stem. The phyllotaxy is called 1/2 rank (distichous) followed by 1/3 and 2/5. Different combinations of all (1/2, 1/3 and 2/5) are termed as mixed type.

- Stipule nature

Stipules are outgrowths from leaf base, which usually protects the young axillary buds- depending on hanging arrangement of stipules nature, grouped into foliaceous, free-lateral and bud scale.

- Stipule duration

The attachment of stipules with leaf base was observed visually and depending on attachment duration, it was grouped into caducous and persistent.

- Leaf lobation type

The leaf lobation was observed visually and grouped primarily into unlobed and lobed. Under lobed group it was again grouped into deeply lobed, medium lobed and shallow lobed.

- Leaf lobation number

The lobation number on a leaf was counted and scored as No lobation (0), lobation with (1–5) and lobation with (6–10).

- Leaf nature

The leaf nature was recorded based on unlobed, lobed and grouped into homophyllous (All the leaves in the plant are of similar nature either entire or lobed), heterophyllous (when some leaves are lobed and some are unlobed either in the same branch or in different branches of the same plant).

- Leaf color

The mature leaf color was observed from each accession on 3 plants and scored visually as green (G) and dark green (DG).

- Leaf surface

The leaf surface was grouped based on feeling by touch on it and grouped into rough, slightly rough and smooth.

- Leaf texture

The leaf texture was grouped based on feeling method and grouped into a) chartaceous, opaque and like writing paper and b) coriaceous, leathery, thick and stiff.

- Leaf apex

It is the portion of leaf bounded by approximately the upper 25% of the leaf margin and based on observation, the leaf apex grouped into a) Acute (pointed and narrow), b) Acuminate (the apex is drawn out into a short tapering tail) and c) Caudate (when the apex shows long tapering tail).

Table 1: List of descriptors for characterization and evaluation

S. No.	Descriptors	Days after pruning (DAP)
1	Morphology	70–90 days
2	Reproductive behavior	Flowering season (April-May and Sept-Oct.)
3	Leaf anatomy	Leaf samples after 60–70 days
4	Growth and yield	70 days
5	Biochemical	60 days
6	Propagation	90 days

- Leaf margin

The margin of the lamina was grouped into a) crenate (margin toothed and teeth are rounded without a pointed apex) b) Serrate (serrations are pointed with their axes inclined to the trend of margin), c) Dentate (if the margin is tooth-like edges), and d) Repand (when the margins form a smooth line or arc without noticeable projections).

- Leaf base

It is the portion of the leaf bounded by approximately lower 25% of the margin. The observations were recorded into a) Cordate, leaf base embayed in a sinus whose sides are straight or convex, b) Truncate, leaf base terminating abruptly as if cut, margin perpendicular to the mid vein and nearly so, c) Lobate, leaf base small to large rounded projections whose inner margins towards the petiole and are in partly concave shape.

- Leaf shape

The leaf shape is ovate in nature which represents the greatest width intersecting the leaf axis basal to the mid point of the later axis and based on (L/W) ratio of leaf it was grouped as Narrow Ovate (2:1), Ovate (1.5:1), Wide Ovate (1.2:1) and Cordate (<1:1).

Leaf Anatomy

- Stomata size

The mature leaf at 5th to 7th position from the longest branch was collected from the shoots of 3 plants and was preserved in FAA (5 mL formalin, 5 mL glacial acetic acid and 90 mL 70% absolute alcohol). Stomatal impression was made by quick fix method from the lower surface of the leaf and observed under microscope. Nine observations were recorded and measured both length and width of stomata in μm .

- Stomatal frequency

Stomatal frequency was recorded in a unit area under microscope in nine observation fields. The number counted in an unit area was expressed in mm^2 .

- Idioblast length

The FAA fixed leaf material was taken and free hand section was made and observed under light microscope. The idioblast projection length protrude from the epidermis was measured. A total of nine observations were recorded in μm .

- Idioblast width

A total of nine observations were made as described in idioblast length.

- Idioblast Frequency

The fixed leaf peeling was made by quick fix method to get the impression of idioblast in the upper surface of leaf. The idioblast frequency was counted in an unit area under microscope in nine microscopic fields and converted as sq. mm .

- Palisade thickness

The ground tissue system, called mesophyll tissues, is often differentiated into columnar palisade parenchyma on the adaxial side of the leaf. A thin free hand section of leaf was made and thickness of the palisade tissues was measured under a microscope. Total 9 observations taken in μm .

- Spongy thickness

The mesophyll tissues consist of irregular or isodiametric spongy parenchyma on the abaxial surface of leaf. A thin free hand section of leaf was made and thickness of spongy tissue was measured under microscope. A total of 9 observations in μm recorded.

- Palisade-Spongy ratio

It is calculated as the value of the palisade/spongy ratio.

- Upper cuticle layer

Epidermal cells have unevenly thickened walls, the outer and radial walls being much thicker than the inner walls. The walls are much cutinised with fatty substance cutin, which forms the cuticle occurring all over the outer wall of the epidermal cells. A thin free hand section of leaf was made and thickness of upper cuticular layer was measured under microscope. A total of 9 observations in μm recorded.

- Lower cuticle layer

The outer layer of the leaf's lower surface (abaxial) is cutinised with fatty substance - cutin, which covers the outer layer of epidermal cells. Thin free hand section of leaf was made and layer's thickness was measured under microscope. A total of 9 observations in μm recorded.

- Upper epidermis

Epidermal tissue system consist of epidermal layers occurring on the leaf's adaxial surface (upper). After making thin free hand section it was observed under microscope and thickness measured in μm for 9 observations.

- Lower epidermis

Epidermal tissue system consists of epidermal layers occurring on the leaf's abaxial surface (lower). After making hand section of preserved leaf, it was observed under microscope and thickness measured in μm for 9 observations.

- Leaf thickness

Total leaf thickness is the addition of palisade, spongy, upper cuticle, lower cuticle, upper epidermis and lower epidermis layers.

- Chloroplast number/stomata

Chloroplast is the most important plastid responsible for carrying out the important functions of photosynthesis. In mulberry the chloroplast number indirectly indicates the ploidy status. Young leaves from 3rd to 5th position leaves from a branch were collected in a polythene bag and preserved in distilled water. The fresh peel from lower leaf surface was cut into pieces, avoiding veinlets and observed under microscope after stained with 2% Potassium Iodide (KI) solution. The chloroplast absorbs the KI solution, becomes dark black in color, and were counted under microscope. A total 9 observations/slide were recorded.

Biochemical

- Chlorophyll-a

The fresh leaves from 7 to 9th position were collected after 60 days of pruning for chlorophyll estimation. The leaf samples were processed following Hiscox and Israelstam (1979) method. The absorbance at 663 and 645 nm was recorded in a spectrophotometer. The amount of chlorophyll-a was calculated as per Arnon (1949).

- Chlorophyll-b

The amount of chlorophyll-b was calculated as per Arnon (1949).

- Total chlorophyll

Total chlorophyll was calculated as per Arnon (1949).

- Chlorophyll- a/b

It is a ratio of chlorophyll a and chlorophyll b.

- Protein content (%)

Nine leaves each from three plants/ accessions at 7th- 9th position were collected after 60 days of pruning. Dried the leaves at 60°C for 72 hours and take the dry weight. Weighed 100 mg of the sample, added 10 mL of 5% TCA, and centrifuged at 10000 rpm for 10 minutes. Discarded supernatant, added 1N NaOH to the pellet, and incubated for 12 hours. Centrifuged at 10000 rpm for 10 minutes and collected the supernatant. Taken 0.2 mL of the aliquot and estimated protein content as per Lowry *et al.* (1951). Read absorbance at 700 nm. Protein content on fresh weight basis was calculated based on moisture content of the leaf and protein content on dry weight basis.

- Carbohydrate content (%)

Prepared the leaf dry powder per the method described similar to protein and took 100 as leaf dry powder. Added 10 mL of 80% hot ethanol, taken the extract, evaporated

in hot water bath and diluted to 10 mL with distilled water. Estimated carbohydrates as per Plumer (1971). Taken 0.2 mL of extract and added 4 mL of 0.2% freshly prepared anthrone reagent. Mixed the solution thoroughly and incubated for 10 minutes in boiling water bath and cool rapidly in water. Read the absorbance at 620 nm. Carbohydrate content on fresh weight basis was calculated based on moisture content of the leaf and carbohydrate content on dry weight basis.

Results

Morphological Characterization of Mulberry Genetic Resources

The variability and frequency distribution of 22 indigenous mulberry accessions for different morphological characters are presented in Tables 2 (a, b) 3. Out of 22 indigenous accessions studied, 14 accessions are erect (63.64%), 5 semi-erect (22.73%), and 3 spreading (13.64%) in nature of growth. The leaf lobation is mostly unlobed (81.82%) followed by medium lobation (13.64%) and shallow lobed (4.55%). All the studied accessions are with chartaceous (thin papery) leaf texture, while only one accession has persistent stipules.

Reproductive Characterization of Mulberry Genetic Resources

The reproductive characters were recorded for 22 accessions during the flowering season (Sept. to Oct. and Feb.-March). The sex expression of the accessions studied indicated preponderance of unisexuality with dioecious nature. Among 22 accessions, 14 are female, 6 male and 2 had both male and female inflorescences Table 4).

Highest variability (CV=97.54%) was recorded in female inflorescence followed by fruit weight (CV=78.38%) for the reproductive parameters among the mulberry accessions studied. The number of male flowers varied from 20–50 and female flowers from 15-101/ catkin. The range, mean and variability for different reproductive parameters are presented in the Table 5.

Leaf Anatomical Characterization of Mulberry Genetic Resources

Total 22 mulberry accessions were characterized for 15 anatomical characters following the method described by Metcalf and Chalk, (1972). Leaves were selected from 9th position and preserved in FAA solution till further study. For counting the number of stomata epidermal peelings of fresh young leaves was used. The variability in respect of leaf anatomical characters is given in Table 6.

The top performing five mulberry accessions for different anatomical parameter and promising accessions based on anatomical parameters are presented in Tables 7 and 8.

Evaluation of Mulberry Genetic Resources for Propagation Traits

Total 22 mulberry accessions were evaluated for propagation traits in RBD with three replications and two check variety. Maximum survival percentage was given in Table 9.

Table 2A: Details of morphological characterization of mulberry genetic resources

S. No.	Acc. No.	Branching nature	Curve or straightness of the branch	Lobation type	Leaf color	Leaf surface
1.	MI-0933	Erect	Straight	Unlobed	Green	Smooth
2.	MI-0934	Erect	Straight	Unlobed	Green	Smooth
3.	MI-0935	Erect	Slightly curved	Unlobed	Green	Smooth
4.	MI-0936	Erect	Straight	Unlobed	Green	Smooth
5.	MI-0937	Erect	Straight	Unlobed	Green	Smooth
6.	MI-0938	Erect	Straight	Medium lobed	Green	Smooth
7.	MI-0939	Erect	Slightly curved	Unlobed	Deep green	Slightly rough
8.	MI-0940	Erect	Straight	Unlobed	Green	Smooth
9.	MI-0941	Semi erect	Slightly curved	Unlobed	Green	Smooth
10.	MI-0942	Spreading	Slightly curved	Unlobed	Green	Smooth
11.	MI-0943	Erect	Straight	Unlobed	Green	Smooth
12.	MI-0944	Spreading	Straight	Unlobed	Green	Smooth
13.	MI-0945	Erect	Straight	Unlobed	Green	Smooth
14.	MI-0946	Erect	Straight	Unlobed	Green	Smooth
15.	MI-0947	Spreading	Straight	Medium lobed	Green	Smooth
16.	MI-0948	Erect	Slightly curved	Shallow lobed	Green	Smooth
17.	MI-0949	Semi erect	Slightly curved	Unlobed	Green	Smooth
18.	MI-0950	Semi erect	Curved	Unlobed	Green	Smooth
19.	MI-0951	Semi erect	Slightly curved	Unlobed	Green	Smooth
20.	MI-0952	Semi erect	Straight	Unlobed	Green	Smooth
21.	MI-0953	Erect	Straight	Unlobed	Green	Smooth
22.	MI-0954	Erect	Straight	Medium lobed	Green	Smooth

Table 2B: Contd...

S. No.	Acc. No.	Phyllotaxy	Leaf color	No. of lobes	Leaf nature	Leaf apex
1.	MI-0933	1/3	Green	0	Homophyllous	Acute
2.	MI-0934	1/2, 2/5, 1/3	Green	0	Homophyllous	Acute
3.	MI-0935	1/3, 1/4	Green	0	Homophyllous	Acuminate
4.	MI-0936	1/2, 1/4	Green	0-4	Homophyllous	Acute
5.	MI-0937	1/2, 1/4	Green	0	Homophyllous	Acuminate
6.	MI-0938	1/2, 1/3	Deep green	0	Homophyllous	Acuminate
7.	MI-0939	1/2, 1/4, 2/5	Green	0	Homophyllous	Acuminate
8.	MI-0940	1/3, 2/5	Green	0	Homophyllous	Acuminate
9.	MI-0941	1/2, 1/4, 1/5	Green	0	Homophyllous	Acuminate
10.	MI-0942	1/3, 1/5, 2/3	Green	0	Homophyllous	Acuminate
11.	MI-0943	1/3, 1/4	Green	0	Homophyllous	Acuminate
12.	MI-0944	1/3, 1/5	Green	0	Homophyllous	Acuminate
13.	MI-0945	1/3, 1/5	Green	0-3	Heterophyllous	Acuminate
14.	MI-0946	1/3	Green	0-2	Heterophyllous	Acuminate
15.	MI-0947	1/3	Green	0	Homophyllous	Acuminate
16.	MI-0948	1/2, 1/3	Green	0	Homophyllous	Acuminate
17.	MI-0949	2/4	Green	0	Homophyllous	Acuminate
18.	MI-0950	1/5, 1/3, 2/4	Green	0	Homophyllous	Acuminate
19.	MI-0951	1/2, 1/3	Green	0-4	Homophyllous	Acuminate
20.	MI-0952	1/3	Green	0	Heterophyllous	Acuminate
21.	MI-0953	2/3	Green	0	Homophyllous	Acute
22.	MI-0954	1/2	Green	0	Homophyllous	Acuminate

Table 3: Frequency table for qualitative descriptors

<i>Characters</i>	<i>Frequency</i>	<i>Percentage</i>	<i>Characters</i>	<i>Frequency</i>	<i>Percentage</i>
<i>Growth nature</i>			<i>Number of lobation</i>		
Erect	14	63.64	0	18	81.82
Semi erect	5	22.73	0-2	1	4.55
Spreading	3	13.64	0-3	1	4.55
Total	22	100.00	0-4	2	9.09
<i>Branching nature</i>			<i>Total</i>	22	100.00
Curved	1	4.55	<i>Leaf nature</i>		
Slightly curved	7	31.82	Heterophyllous	4	18.18
Straight	14	63.64	Homophyllous	18	81.82
Total	22	100.00	Total	22	100.00
<i>Color of young shoot</i>			<i>Leaf color</i>		
Green	20	90.91	Deep green	1	4.55
Light green	2	9.09	Green	21	95.45
Total	22	100.00	Total	22	100.00
<i>Color of mature stem</i>			<i>Leaf texture</i>		
Greenish brown	7	31.82	Chartaceous	22	100.00
Greenish grey	2	9.09	Total	22	100.00
Grey green	13	59.09	<i>Leaf apex</i>		
Total	22	100.00	Acuminate	19	86.36
<i>Stipule duration</i>			<i>Acute</i>	3	13.64
Caducous	21	95.45	Total	22	100.00
Persistent	1	4.55	<i>Leaf base</i>		
Total	22	100.00	Cordate	20	90.91
<i>Stipule nature</i>			Truncate	2	9.09
Foliaceous	1	4.55	Total	22	100.00
Free-lateral	21	95.45	<i>Leaf margin</i>		
Total	22	100.00	Serrate	22	100.00
<i>Lobation type</i>			Total	22	100.00
Medium lobed	3	13.64			
Shallow lobed	1	4.55			
Unlobed	18	81.82			
Total	22	100.00			

Table 4: Details of reproductive characterization of mulberry genetic resources

<i>S. No.</i>	<i>Acc. No.</i>	<i>Sex</i>	<i>Inflorescence length (cm)</i>		<i>Inflorescence width (cm)</i>		<i>No. of flowers</i>		<i>Style length</i>	<i>Stigma length</i>
			♂	♀	♂	♀	♂	♀		
	MI-0933	MLFL	2.30	1.64	0.62	0.54	32.40	54.60	1.12	2.00
	MI-0934	FEML	--	1.73	--	0.45	--	45.00	0.23	3.65
	MI-0935	FEML	--	1.98	--	0.68	--	64.00	0.68	4.16
	MI-0936	FEML	--	1.80	--	0.56	--	41.40	0.74	4.48
	MI-0937	FEML	--	1.58	--	0.50	--	60.80	0.36	3.62
	MI-0938	FEML	--	9.66	--	0.48	--	101.20	0.72	3.22
	MI-0939	FEML	--	1.36	--	0.52	--	26.00	0.50	4.90
	MI-0940	FEML	--	1.50	--	0.60	--	24.40	1.20	4.84
	MI-0941	FEML	--	1.06	--	0.40	--	15.40	1.42	4.28

MI-0942	FEML	--	1.66	--	0.52	--	37.00	1.06	4.64
MI-0943	FEML	--	2.14	--	0.56	--	44.40	1.30	4.50
MI-0944	MALE	1.74	--	0.46	--	19.60	--	--	--
MI-0945	MALE	2.73	--	0.58	--	37.20	--	--	--
MI-0946	MLFL	3.38	1.88	0.56	0.56	49.80	62.40	0.50	3.80
MI-0947	FEML	--	1.18	--	0.56	--	42.60	0.24	2.48
MI-0948	MALE	2.26	--	0.48	--	33.80	--	--	--
MI-0949	MALE	3.18	--	0.48	--	40.60	--	--	--
MI-0950	FEML	--	1.98	--	0.70	--	90.20	0.66	3.06
MI-0951	MALE	3.44	--	0.72	--	48.80	--	--	--
MI-0952	MALE	2.10	--	0.42	--	22.80	--	--	--
MI-0953	FEML	--	1.34	--	0.48	--	33.00	0.42	2.76
MI-0954	FEML	--	1.04	--	0.46	--	17.00	1.08	4.82

Table 5: Variability study in mulberry genetic resources for reproductive parameters

Parameters	Min.	Max.	Mean $\pm$ SE	CV%
Female inflorescence length (cm)	1.04	9.66	2.09 $\pm$ 0.53	97.56
Female inflorescence breadth (cm)	0.40	0.70	0.54 $\pm$ 0.02	14.76
Male inflorescence length (cm)	1.74	3.44	2.65 $\pm$ 0.24	24.14
Male inflorescence breadth (cm)	0.42	0.72	0.54 $\pm$ 0.04	18.76
No. of flowers/catkin (Female)	19.60	49.80	35.63 $\pm$ 4.13	30.69
No. of flowers/catkin (Male)	15.40	101.20	47.46 $\pm$ 6.25	50.98
Style length (mm)	0.23	1.42	0.76 $\pm$ 0.10	50.45
Stigma length (mm)	2.00	4.90	3.83 $\pm$ 0.23	23.71
Fruit length (cm)	1.02	1.20	2.11 $\pm$ 0.26	42.33
Fruit diameter (cm)	0.40	1.00	0.68 $\pm$ 0.07	33.92
Fruit peduncle length (cm)	0.20	1.49	0.51 $\pm$ 0.08	54.71
Fruit weight (g)	0.13	4.90	0.54 $\pm$ 0.12	78.38

Table 6: Details of anatomical characterization of mulberry genetic resources

Parameters	Min.	Max.	Mean	CV%
Stomatal size (sq.µm)	204.30	315.87	256.60	12.84
Stomatal frequency/sq.mm	418.04	1102.30	779.76	27.26
No. of chloroplasts/stomata	8.00	17.20	12.02	21.06
Idioblast length (µm)	15.17	23.45	18.33	12.16
Idioblast diameter (µm)	17.24	26.90	21.09	13.41
Idioblast frequency/sq.mm	8.35	31.23	15.54	39.11
Cystolith length (µm)	37.24	80.29	57.64	20.16
Cystolith breadth (µm)	44.83	74.48	59.65	13.98
Upper cuticle thickness (µm)	6.21	12.41	8.28	23.03
Lower cuticle thickness (µm)	3.45	6.90	4.02	27.19
Upper epidermis thickness (µm)	17.24	24.83	20.86	10.65
Lower epidermis thickness (µm)	6.21	11.03	7.87	20.95
Palisade thickness (µm)	58.62	88.96	68.05	15.07
Spongy thickness (µm)	64.14	87.58	71.32	10.94
Leaf thickness (µm)	164.82	217.24	180.40	9.26
Palisade spongy ratio	0.81	1.32	0.97	13.36

The mean values for stomatal size ranged from 204.30–315.87 sq. µm. the stomatal frequency ranged from 418.04–1102.30 per sq.mm and leaf thickness from 164.82–217.24 µm. maximum variability was observed in idioblast frequency/ sq. µm (CV= 39.11%) followed by stomatal frequency 27.26%, whereas minimum variability was observed for stomatal width (7.05%), length (7.33%) and leaf thickness (9.26%) among different anatomical characters recorded in the 22 mulberry accessions.

Table 7: Top performing accessions for anatomical characters

Parameters	Range	Top five accessions
Stomatal size (μm)	174.46–224.96	MI-0933, MI-0954, MI-0953, MI-0940, MI-0941
Stomatal frequency (mm^2)	345.25–519.95	MI-0955, MI-0946, MI-0949, MI-0946, MI-0947
Idioblast length (μm)	15.17–16.55	MI-0949, MI-0952, MI-0948, MI-0951, MI-0953
Idioblast frequency/sq.mm	8.35–12.23	MI-0950, MI-0955, MI-0933, MI-0937, MI-0940
Palisade tissue thickness (μm)	68.96–88.96	MI-0946, MI-0948, MI-0951, MI-0953, MI-0942
Spongy tissue thickness (μm)	73.10–87.58	MI-0948, MI-0935, MI-0951, MI-0953, MI-0954
Palisade spongy ratio	1.02–1.32	MI-0946, MI-0947, MI-0956, MI-0942, MI-0954
Upper cuticle thickness (μm)	10.35–18.62	MI-0947, MI-0935, MI-0942, MI-0952, MI-0950
Lower cuticle thickness (μm)	4.83–6.90	MI-0945, MI-0952, MI-0937, MI-0953, MI-0933
Upper epidermis thickness (μm)	24.83–42.76	MI-0935, MI-0946, MI-0940, MI-0952, MI-950
Lower epidermis thickness (μm)	8.28–11.04	MI-0946, MI-0951, MI-0953, MI-0948, MI-0938
Leaf thickness (μm)	193.79–217.24	MI-0946, MI-0951, MI-0953, MI-0947, MI-0939
No. of chloroplasts/stomata	13.60–17.2	MI-0951, MI-0953, MI-0945, MI-0940, MI-0935

Table 8: Top performing accessions for anatomical characters

Acc. No.	No. of traits	Trait number (value)
MI-0945	8	2 (418.04), 3 (15.17), 4 (8.34), 6 (73.10), 8 (11.03), 10 (24.82), 11 (8.27), 13 (17.20)
MI-0946	7	2 (345.24), 3 (15.17), 8 (18.62), 9 (6.89), 10 (42.75), 11 (11.03), 12 (202.06)
MI-0952	5	1 (219.45), 2 (476.27), 5 (69.65), 6 (82.06), 12 (193.78)
MI-0951	4	5 (88.96), 7 (1.32), 11 (11.03), 12 (202.44)
MI-0936	4	5 (75.17), 6 (83.44), 12 (195.85), 13 (14.40)
MI-0950	4	8 (10.34), 2 (519.95), 7 (1.02), 10 (24.82)
MI-0948	4	1 (204.30), 7 (1.06), 8 (11.03), 10 (31.72)

1. Stomatal size (μm), 2. Stomatal frequency (mm^2), 3. Idioblast length (μm), 4. Idioblast frequency (mm^2), 5. Palisade tissue thickness (μm), 6. Spongy tissue thickness (μm), 7. Palisade:Spongy ratio (μm), 8. Upper cuticle thickness (μm), 9. Lower cuticle thickness (μm), 10. Upper epidermis thickness (μm), 11. Lower epidermis thickness (μm), 12. Leaf thickness (μm), 13. Number of chloroplasts/stomata.

Among the characters studied, highest coefficient of variation (CV%) was observed in root weight (37.76%) followed by root shoot ratio and percent survival (Table 10). The top performing mulberry accessions for different propagation traits and based on the multiple propagation traits are presented in Table 11. The accessions MI-0953 and MI-0948 proved to be promising for propagation parameters.

Evaluation of Mulberry Genetic Resources for Growth and Yield Parameters

A twenty two mulberry accessions were studied for growth and yield parameters and maximum variability was observed in biomass (62.75%) and leaf yield (62.09%), while lowest variability was observed for moisture content of the leaves (Table 12).

The top performing mulberry accessions for individual growth and yield traits and multiple growth and yield traits are presented in Tables 13 and 14, respectively. The accessions MI-0952 and MI-0948 proved to be promising for growth and yield parameters.

Evaluation of Mulberry Genetic Resources for Biochemical Parameters

Leaf anatomical status plays an important role in growth and development of silkworm larvae and in turn cocoons production per unit area. Total 22 mulberry accessions were evaluated for important biochemical parameters that contribute to leaf quality and variability for different parameters which is presented in Table 15. The results indicate that the variability was medium for different biochemical parameters among the 22 accessions evaluated. The parameter-wise and multiple trait based top performing accessions are indicated in Tables 16 and 17. Among the mulberry accessions studied MI-0935, MI-0948 and MI-0938 are promising based on biochemical analysis.

Discussion

Characterization and evaluation of any crop is a continuous process to evolve new varieties suitable for commercial utilization. Studies have indicated that the variation of important aboveground and underground agronomic traits

Table 9: Survival percentage of mulberry accessions

Survival (%)	No. of Accns	Accessions
> 90	3	MI-0953, MI-0948, MI-0938
80–90	4	MI-0951, MI-0947, MI-0952, MI-0936
< 60	8	MI-0940, MI-0939, MI-0941, MI-0933, MI-0954, MI-0950, MI-0946, MI-0935
No survival	7	MI-0934, MI-0937, MI-0945, MI-0943, MI-0949, MI-0942, MI-0944
Total	22	

Table 10: Variability for propagation parameters

Parameters	Min.	Max.	Mean	SE	CV%
Survival %	20.00	96.67	76.67	8.27	32.34
Shoot length (cm)	30.89	52.56	42.66	2.65	18.65
Number of roots	2	4	3	0.2	20.00
Longest root length (cm)	17.00	25.11	20.52	0.97	14.11
Dry root weight (g)	0.40	1.25	0.80	0.1	37.76
Root volume (ml)	0.22	0.49	0.33	0.02	22.00
Root shoot ratio	0.10	0.27	0.15	0.02	33.20

Table 11: Top performing mulberry accessions based on multiple traits

Accessions	Traits	Trait number (value)
MI-0953	6	1 (91.67), 2 (3), 3 (25.11), 5 (0.99), 6 (0.16)
MI-0948	5	2 (3.44), 3 (24.67), 4 (1.25), 5 (0.50), 6 (0.27)
MI-0938	4	3 (21.22), 4 (1.15), 5 (0.33), 6 (0.27)
MI-0951	4	1 (88.33), 2 (4.22), 4 (0.99), 6 (0.19)
MI-0947	3	2 (3.56), 3 (21.24), 6 (0.34)

1. Survival %; 2. Number of roots; 3. Longest root length (cm); 4. Dry root weight (g); 5. Root volume (mL); 6. Root shoot ratio.

Table 12: Variability for growth and yield parameters

Parameters	Min.	Max.	Mean	SE	CV%
Number of shoots	8.33	28.67	15.94	1.83	38.14
Length of longest shoot (cm)	140.00	239.33	185.75	8.72	15.57
Length of lamina (cm)	10.67	24.67	15.58	1.12	23.75
Width of lamina (cm)	7.00	16.00	10.40	0.73	23.32
Weight of lamina (g)	0.92	4.97	2.40	0.34	46.82
Total shoot length (cm)	1075.33	4086.33	2065.28	280.94	45.12
Leaf yield/plant (kg)	0.45	2.20	0.92	0.17	62.09
Inter-nodal distance (cm)	3.26	8.28	4.84	0.37	25.21
Weight of 100 leaves (g)	106.07	614.50	270.97	42.94	52.56
Moisture content of leaves (%)	61.50	74.20	66.03	1.03	5.15
Moisture content after 6 hrs (%)	51.14	65.92	56.17	1.48	8.71
Moisture retention capacity (%)	54.14	78.17	66.21	2.09	10.47
Biomass/plant (kg)	0.92	4.10	2.28	0.43	62.75

Table 13: Top performing mulberry accessions for growth and yield traits

Traits	Range	Accessions
Number of shoots	17.33–28.67	MI-0936, MI-0941, MI-0948, MI-0951, MI-0952
Length of longest shoot (cm)	184.33–239.33	MI-0936, MI-0938, MI-0952, MI-0942, MI-0954
Length of lamina (cm)	17.67–24.67	MI-0951, MI-0952, MI-0936, MI-0943, MI-0950
Width of lamina (cm)	11.83–16.00	MI-0933, MI-0940, MI-0948, MI-0952, MI-0938

Total shoot length (cm)	2154–4086	MI-0949, MI-0936, MI-0952, MI-0944, MI-0933
Leaf yield/plant (kg)	0.97–2.2	MI-0944, MI-0936, MI-0949, MI-0942, MI-0936
Inter-nodal distance (cm)	3.19–4.05	MI-0952, MI-0938, MI-0950, MI-0949, MI-0944
Weight of 100 leaves (g)	43.00–61.5	MI-0933, MI-0949, MI-0953, MI-0950, MI-0938
Moisture content of leaves (%)	68.16–74.20	MI-0949, MI-0944, MI-0948, MI-0933, MI-0953
Moisture content after 6 hrs (%)	60.37–65.92	MI-0951, MI-0953, MI-0944, MI-0947, MI-0950
Moisture retention capacity (%)	72.16–78.17	MI-0949, MI-0950, MI-0938, MI-0950, MI-0954
Biomass/plant (kg)	2.3–4.40	MI-0941, MI-0948, MI-0933, MI-0954, MI-0938

Table 14: Top performing mulberry accessions based on multiple traits

Acc. No.	No. of Traits	Traits (Value)
MI-0936	6	1 (22), 2 (239.33), 4 (11.83), 6 (3633.67), 6 (1.29), 12 (4.31)
MI-0952	5	1 (28.67), 2 (206), 5 (4086.33), 6 (0.967), 12 (3.61)
MI-0933	5	2 (196.67), 5 (217.33), 6 (1.79), 7 (3.26), 12 (4.12)
MI-0944	5	1 (21.33), 5 (2248.66), 9 (68.16), 10 (60.84), 11 (72.65)
MI-0949	5	2 (184.33), 3 (18), 9 (68.77), 10 (63.26), 11 (78.17)

1. Number of shoots; 2. Longest root length (cm); 3. Length of lamina (cm); 4. Width of lamina (cm); 5. Total shoot length (cm); 6. Leaf yield/plant (kg); 7. Inter-nodal distance (cm); 8. Weight of 100 leaves (g); 9. Moisture content of leaves (%); 10. Moisture content after 6 hrs (%); 11. Moisture retention capacity (%); 12. Biomass/plant (kg).

Table 15: Variability of mulberry accessions for biochemical parameters

Parameter	Mean	Min.	Max.	SE	CV %
Chlorophyll a (mg/g fr. wt.)	1.64	1.08	2.00	0.06	17.33
Chlorophyll b (mg/fr. wt.)	0.27	0.17	0.36	0.01	21.95
Total chlorophyll (mg/fr. wt.)	1.91	1.27	2.35	0.07	16.66
Chlorophyll a/b ratio	6.4	4.17	9.32	0.29	21.71
Protein (% dry wt.)	8.43	5.52	12.34	0.35	19.75
Water soluble carbohydrates (% dry wt.)	13.15	8.96	17.94	0.54	19.24

Table 16: Top performing mulberry accessions for biochemical parameters

Parameter	Range	Accessions
Chlorophyll a (mg/g fr. wt.)	1.90–2.00	MI-0935, MI-0950, MI-0948, MI-0944, MI-0938
Chlorophyll b (mg/fr. wt.)	0.34–0.36	MI-0950, MI-0949, MI-0948, MI-0952, MI-0955
Total chlorophyll (mg/fr. wt.)	2.18–2.35	MI-0950, MI-0948, MI-0944, MI-0935, MI-0951
Chlorophyll a/b ratio	7.96–9.32	MI-0940, MI-0936, MI-0935, MI-0937, MI-0938
Protein (% dry wt.)	9.47–12.34	MI-0935, MI-0948, MI-0938, MI-0952, MI-0954
Water soluble carbohydrates (% dry wt.)	15.65–17.94	MI-0951, MI-0947, MI-0952, MI-0938, MI-0935

Table 17: Top performing mulberry accessions based on multiple traits

Acc. No.	No. of Traits	Traits (Value)
MI-0935	5	1 (200), 3 (2.24), 4 (8.29), 5 (12.34), 6 (15.65)
MI-0948	4	1 (1.94), 2 (0.35), 3 (2.29), 5 (11.88)
MI-0938	4	1 (1.90), 4 (7.96), 5 (11.38), 6 (16.22)

1. Chlorophyll a (mg/g fr. wt.); 2. Chlorophyll b (mg/g fr. wt.); 3. Total chlorophyll (mg/g fr. wt.); 4. Chlorophyll a/b ratio; 5. Protein (% dry wt.); 6. Water soluble carbohydrates (% dry wt.).

in mulberry is largely continuous in nature (Vijayan 2008). Ananda Rao *et al.* (2011) have studied the variation among different mulberry genetic resources for morpho-reproductive characters. They identified promising drought tolerant lines and potential areas based on the variation and abundance of mulberry genetic resources for *in-situ*

conservation. Jayeoba *et al.* (2014) characterized eight mulberry varieties using morphological and chemical parameters and recommended K2, S34, S30 and S54 as promising varieties. The exotic mulberry accessions were evaluated for growth and yield traits by Tikader and Kamble (2009). The study showed maximum coefficient

of variation in leaf yield per plant followed by total shoot length and leaf moisture content. Similarly in the present study highest coefficient of variation was observed in leaf yield per plant (62.09%). Suresh *et al.* (2018) conducted a multivariate analysis of indigenous and exotic mulberry germplasm and identified diverse genotypes as promising donors in improving foliage production. Tikader and Kamble (2008) have studied the variation in indigenous mulberry germplasm on growth and yield and identified better performing accessions. Our study also highlighted the variation of indigenous accessions among various parameters.

Conclusion

Germplasm is a valuable natural resource that provides knowledge about the genetic composition of a particular species and is crucial for conserving plant diversity. Mulberry breeders will require much genetic diversity to select and recombine favorable traits through cross breeding. In this context the study was conducted and the performances of indigenous mulberry accessions were evaluated statistically. The accessions MI-0946, MI-0945, MI-0953, MI-0948, MI-0935, MI-0948, MI-0936 and MI-0952 were identified as top performers based on multiple trait analysis. These accessions can serve as promising donors for future breeding program for the development of improved varieties in mulberry.

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RESEARCH ARTICLE

Assessment of Wheat Genotypes for Spot Blotch and Other Traits under Normal and Diseased Conditions

Rashmi K. Reddy and Ravindra Prasad*

Abstract

Exploring the existing variability among wheat germplasm lines for trait of interest is of utmost importance for plant breeders to start the plant breeding activity under crop improvement programme. Hence, with this aim, 93 wheat genotypes were evaluated for spot blotch resistance and other traits in normal and artificial epiphytotic conditions during *rabi*, 2018-19 at Agriculture Research Farm, Institute of Agricultural Sciences, Banaras Hindu University. Findings revealed the presence of considerable variability among the genotypes for the studied traits grown in two different conditions hence, there is ample scope to develop superior genotypes along with spot blotch disease resistance. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) shows the minute difference for area under disease progress curve (AUDPC) and other traits in both conditions, indicating low environmental influence. High heritability with high genetic advancement for spot blotch disease resistance specified that its inheritance pattern may be additive in nature; therefore, direct selection could be effective in a breeding programme. In both conditions, genotype Raj 3814 had the lowest disease severity; in each environment, we identified 10 highly resistant genotypes. Thus, these genotypes can be used as resistant parent to breed the new source of resistance along with better yield. A negative correlation of AUDPC with seed yield per plant and other traits implies indirect selection of these traits to minimize spot blotch disease severity and increase crop yield.

Keywords: Wheat, Spot Blotch, AUDPC, *Bipolaris sorokiniana*, Resistance.

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Introduction

Among the major cereal crops, wheat (*Triticum aestivum* L.) is considered as one of the most paramount food crops in the world (Alzaayid and Aloush, 2021) as it is widely grown and also consumed as a food by huge people in the world. It is considered a staple food for more than 35% of the world's population (FAO, 2018) and because of the rapid population growth, the demand for wheat is expected to be high in 21st century as well (Prasad, 2022). Wheat is a major food crop in the world but is challenged by biotic and abiotic factors, which significantly lowered the crop yield (Kaur *et al.*, 2021). In continuation, wheat crop is challenged by an important fungal disease viz., spot blotch which is causing by *Bipolaris sorokiniana* (Prasad, 2022 and Chandra *et al.* 2019). According to Kumar *et al.* (2019) it is known as the most crucial disease mostly in warm and humid region of South Asia and South America and this disease considerably reduces the crop yield, affect the quality of the crops in the country, especially in the Eastern Gangetic Plains of South Asia, which includes India, Nepal, and Bangladesh (Joshi *et al.* 2007a and Gupta *et al.* 2018). It remains more severe in the north eastern plain zone under late sown conditions where rice-wheat cropping system is followed. In South Asia, around 10 mha of land is affected by spot blotch, of which 9 mha exists in India alone, where rice-wheat cropping system has dominated since the time of green revolution (Joshi *et al.*, 2007a). In severe conditions, disease may

limits the yield upto 18 to 50% in the warmer and humid regions of the world (Gurung *et al.* 2014) and further yield loss could be reached up to 100% under more severe/favorable conditions, if genotypes are lacking the resistance genes for spot blotch disease (Mehta, 1994 and Gupta *et al.* 2018). As per Yadav *et al.* (2015), around 25 million hectare of wheat land is badly affected by spot blotch disease, in continuation, spot blotch disease is known as one of the major constraints for wheat production in the globe, mainly the areas which is tagged as hot and humid climate (Tomar *et al.* 2021).

Severe condition of spot blotch disease is easily can be control by using fungicide but its repeated application involves additional cost to the growers, health hazard and the emergence of fungicidal resistance in the target pathogen. Further, there is still an unavailability of completely resistant wheat genotypes to be cultivated to save the yield loss occurred by spot blotch because it is controlled by many genes (Gupta *et al.* 2018) and tagging the linked genes and its incorporation into single genotype to ensure the high level of resistance (Kumar *et al.* 2019). Therefore, exploring the available diverse germplasm lines and developing the resistance genotypes to save the yield loss that occurred by spot blotch is one of the eco-friendly approaches and continuing process (Prasad *et al.* 2013).

In this context, germplasm lines of wheat have been evaluated intensively by the researchers to find out the efficient donors or genotypes to be used in crossing program and further to obtain desirable segregants in later generations (Lamalaksmi *et al.*, 2013). In recent past, resistance genotypes/genetic stocks have been identified by CIMMYT (Chowdhury *et al.* 2013 and Kumar *et al.*, 2016), Prasad *et al.* (2013) in barley. Similarly, Kumari *et al.* (2018) also identified and reported some of the new source of resistance in wheat crop by screening the genotypes grown across the location and over the years; however, it is not up to the mark. So, screening of diverse wheat genotypes/germplasm for resistance to spot blotch and other desirable traits at large scale is a continuous process in field condition is vital because evaluation of huge genotypes under controlled conditions are not feasible (Duveiller and Sharma 2012, Lillemo *et al.* 2013 and Tomar *et al.* 2021). As per available reports, a number of genotypes are developed with better resistance to spot blotch disease, but this level of resistance is not enough to minimize yield loss because of slow progress and the polygenic nature of trait (Joshi *et al.* 2004b). Besides this, some morphological markers such as leaf angle, leaf wax and stay green have been reported which shows a positive association with spot blotch disease resistance hence, such reports could help to breeders for selecting/developing resistance genotypes with better yielding during the breeding programme (Joshi *et al.* 2007b; Joshi and Chand (2002) and Prasad *et al.* (2013). Various genetic parameters, such as mean, range, and heritability, are used to measure the degree of genetic variability and know the genetic

contribution of genotypes associated with spot blotch resistance is useful and practiced in crop improvement programme (Singh *et al.* 2007, Thakur *et al.* 2018). High heritability for a trait of interest shows the presence of additive gene action hence, and selection may be fruitful for crop improvement of these traits (Thakur *et al.* 2018).

It is therefore, by considering the above facts, experiment was designed to explore the genetic information of a set of 93 diverse wheat genotypes grown in normal as well as in artificial epiphytotic condition in order to tag the superior genotypes having satisfactory yield, resistance and better other desirable traits for its utilization in plant breeding and producing the enough food grains for supplying the demand of growing population.

Materials and Methods

A set of 93 diverse wheat genotypes, comprised of national genetic stock nursery lines and released varieties for different zones viz., North Eastern Plain Zone, North Western Plain Zone, Central Zone, Peninsular Zone and few exotic lines maintained by breeders department of Genetics and Plant Breeding, were screened for spot blotch disease, yield and other traits grown under normal as well as artificial epiphytotic conditions. The experiment was carried during *rabi*, 2018-19 at Agriculture Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi in randomized complete block design. Each genotype was sown in a two rows of 2 m length in three replications in each condition and recommended agronomic practices were followed to raise the healthy crops.

Artificial Epiphytotic Conditions and Disease

Assessment

Spore suspension of 10^4 spore/mL of *Bipolaris sorokiniana* fungus was uniformly sprayed at the time ear emergence Zadoks *et al.* (1974) during evening hours following the method of Chaurasia *et al.* (1999). After inoculation, experimental plots were irrigated immediately to provide a friendly environment of high humidity so that the pathogen can grow and multiply on the host (Joshi and Chand, 2002). The double-digit scoring (DD = 00-99) method was used to record the disease progress (Saari and Prescott, 1975) at three different growth stages of plant viz., GS 63 (beginning of anthesis to half-complete), GS 69 (anthesis complete) and GS 77 (late milking) following the Zadoks *et al.* (1974). The growth pattern (pure culture), conidia morphology of *B. sorokiniana* and disease symptoms (increasing order) are presented in Figure 1(a-c), respectively.

Data Recording and Statistical Analysis

Randomly five plants were tagged for each genotype in all the replication and data for the traits like days to 50% flowering, days to 75% maturity, grain filling duration, plant

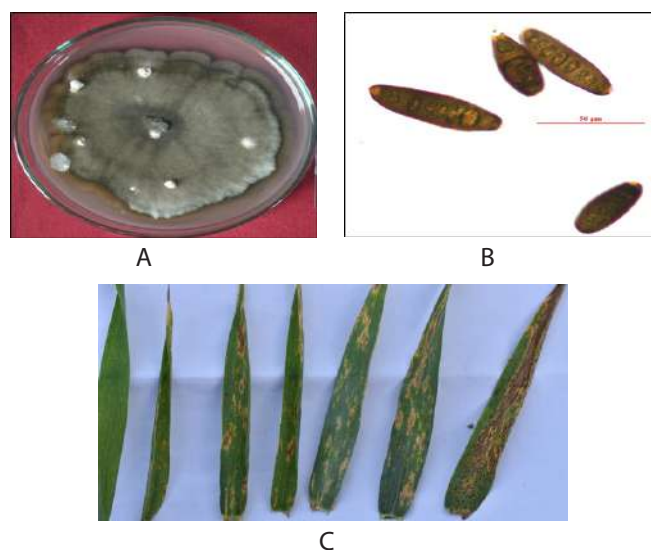


Figure 1 (A): Pure culture of *Bipolaris sorokiniana* grown over PDA, (B) Spore of *B. sorokiniana* causing spot blotch disease in wheat, (C) Spot blotch disease severity on leaves in increasing order (left to right)

height, tillers per plant, peduncle length, spike length, awn length, biological yield per plant, seed yield per plant, harvest index, 1000 grain weight, and disease severity on plot basis was recorded for calculating the AUDPC. The visual assessment of leaf angle and leaf waxiness was also assessed with aim to know its frequency among the population as these traits are associated with resistance to spot blotch. The disease severity in percent was calculated using double-digit score (Roelfs *et al.*, 1992) to estimate the AUDPC.

The mean AUDPC was used to classify the 93 genotypes into different categories, i.e., resistant, moderately resistant, susceptible and AUDPC was calculated using following formula.

$$\text{AUDPC} = \sum_{i=1}^n \left[\frac{(Y_i + Y_{i+1})}{2} \times (t_{i+1} - t_i) \right]$$

where, Y_i = disease level at time t_i

$t_{i+1} - t_i$ = Time (days) between two disease scores

n = number of dates on which spot blotch was recorded

Leaf Angle and Waxiness

Leaf angle was measured just after an ear emergence (Nigam and Srivastava, 1976) using a protractor at the growth stage of 51 to 55 days (Zadoks *et al.* 1974). The genotypes were grouped into four classes like erect, semierect, semi-drooping. The leaf waxiness was recorded at growth stage 69 (Zadokset *et al.* 1974). Each genotype was grouped into waxy, semi-waxy, or non-waxy based on visual appearance of wax on the plant surface/leaf sheath (Prasad *et al.* 2013). The recorded data for 13 traits of two environments were subjected for statistical analysis using INDOSTAT Ver. 9.2 software.

Results and Discussion

Since spot blotch disease of wheat is one of the major constraints to global wheat production, mainly for hot and humid climate growing zones (Tomar *et al.* 2021) and it significantly limits the crop yield (Gupta *et al.* 2018) hence, the present study was designed with aim to explore genetic information of a set of 93 wheat genotypes and its use in plant breeding in order to develop the resistance/durable genotypes and save the yield loss of wheat crops through very effective and eco-friendly approach (Prasad *et al.* 2013, Gupta *et al.* 2018). The analysis of variance (ANOVA) showed significant differences for thirteen traits in 93 wheat genotypes studied under normal and artificial epiphytotic conditions (Tables 1 and 2). These findings indicate the presence of an enormous amount of phenotypic variability among the wheat genotypes for spot blotch disease, yield and yield contributing traits. Its exhibits that there is ample scope to breed the superior genotypes having low disease severity and desirable for other economically important traits by exploring such genotypes as parents donors or cultivars in future breeding programmes.

The estimate of 13 traits of 93 genotypes (grown in normal and artificial condition) are presented in Tables 3 and 4. Which gives an idea about the relative importance of heritable and non-heritable variation (Thakur *et al.* 2018) while the environmental coefficient of variation gives an idea about non-heritable and non-fixable which is of non-significance in plant breeding. Present findings showed that the PCV value was slightly higher than the corresponding GCV for studied traits in both conditions. This indicates that appearance of variation among the genotypes for such traits is not only due to genetic makeup but also environmental effect. Moderate phenotypic variability was observed for traits such as 1000 grain weight, plant height, tillers per plant and peduncle length. Therefore, it is concluded that PCV and GCV for a majority of traits are moderate to high, which depicts considerable variation among 93 genotypes. Hence, exploitation of these traits in future breeding programmes can be done to enhance the crop output (Thakur *et al.* 2018; Thapa *et al.* 2018 ; and Chandra *et al.* (2019). The remaining traits, like days to 50% flowering, days to 75% maturity, grain filling duration and harvest index, exhibited less phenotypic variation, indicating that the environment less influences these traits.

The estimate of heritability and genetic advance also estimated to understand the inheritance pattern of spot blotch disease resistance and other traits of 93 genotypes grown in two environments. Studied characters exhibited higher heritability, accompanied by high genetic advance as percent of mean was also estimated for most of the characters viz seed yield per plant, 1000 grain weight, area under disease progress curve (AUDPC) and other traits. Our results indicate that high heritability of these traits could be

Table 1: Analysis of variance of 93 wheat genotypes for 13 traits grown under normal condition

Sources of variation	DF	DM	GFD	PH	NTPP	PED L.	SL	AL	BYPP	SYPP	HI	1000GW	AUDPC
Replicates	63.78	21.42	15.32	35.91	28.52	39.65	21.79	4.62	84.39	31.07	59.18	17.37	1521.15
Genotypes	32.43**	36.67**	17.87**	347.86**	7.26**	28.10**	4.04**	5.03**	33.76**	7.25**	18.84**	304.647**	26284.24**
Error	0.83	1.26	1.97	2.69	0.53	0.37	0.32	0.28	1.30	0.26	2.12	0.41	950.59
Mean	75.02	113.73	38.71	93.38	9.34	16.67	9.77	5.72	25.09	10.33	41.13	40.18	330.83
C.V.	1.22	0.99	3.63	3.76	4.80	3.65	5.86	5.34	7.55	8.99	3.54	1.50	13.32

Table 2: Analysis of variance of 93 wheat genotypes for 13 traits grown under artificial epiphytotic condition

Sources of Variation	DF	DM	GFD	PH	NTPP	PED L.	SL	AL	BYPP	SYPP	HI	1000GW	AUDPC
Replicates	60.52	60.24	37.60	59.40	37.09	28.47	22.60	9.28	94.04	10.28	12.13	26.90	3245.10
Genotypes	32.31**	43.03**	12.68**	331.34**	6.88**	25.71**	3.46**	5.92**	38.72**	8.07**	15.70**	56.69**	47680.93**
Error	0.88	1.35	2.12	3.09	0.49	0.68	0.35	0.18	1.48	0.36	1.800	0.30	1428.34
Mean	75.89	112.60	36.71	94.34	8.66	17.05	9.86	5.49	24.64	10.08	40.86	39.87	444.20
C.V.	1.24	1.03	3.97	3.19	5.17	4.86	6.01	4.77	8.94	7.97	3.28	1.40	15.51

*, ** Significant at 5 and 1% levels, respectively, CV=Coefficient of Variation (%)

Where, DF = Days to 50 % Flowering, DM = Days to 75 % Maturity, GFD = Grain Filling Duration (days), PH = Plant Height (cm), NTPP = Number of Tillers Per Plant, PED. L. = Peduncle Length (cm), SL= Spike Length (cm), AL = Awn Length (cm), BYPP = Biological Yield Per Plant (gm), SYPP = Seed Yield Per Plant (gm), HI = Harvest Index

Table 3: Estimation of genetic parameters of 93 wheat genotypes for 13 traits grown under normal condition

Parameters	DF	DM	GFD	PH	NTPP	PED L.	SL	AL	BYPP	SYPP	HI	1000 GW	AUDPC
Average Mean	75.02	113.73	38.71	93.38	9.34	16.67	9.77	5.72	25.09	10.33	41.13	40.18	257.78
Lowest Range	67.67	102.00	33.00	74.69	6.47	6.30	6.42	3.06	14.68	5.86	33.50	27.57	141.15
Highest Range	85.00	125.00	44.00	129.12	14.00	26.55	12.70	10.76	34.57	15.21	48.71	52.40	826.75
PCV (%)	4.49	3.18	6.97	11.62	17.83	18.60	12.81	23.90	13.88	21.60	6.75	11.47	29.30
GCV (%)	4.33	3.02	5.95	11.49	16.04	18.24	11.39	22.00	13.11	20.78	5.74	11.37	27.78
ECV (%)	1.22	0.99	3.63	1.76	7.80	3.65	5.86	9.34	4.55	7.99	3.54	1.49	9.32
h ² (Broad Sense)	0.93	0.90	0.73	0.98	0.81	0.96	0.79	0.85	0.89	0.90	0.72	0.98	0.90
Genetic Adv. as % of mean	8.58	5.92	10.46	23.39	29.71	36.84	20.87	41.72	25.52	28.85	10.06	23.23	54.248

Table 4: Estimation of genetic parameters of 93 wheat genotypes for 13 traits grown under artificial epiphytotic condition

Parameters	DF	DM	GFD	PH	NTPP	PED L.	SL	AL	BYPP	SYPP	HI	1000GW	AUDPC
Mean	75.89	112.60	36.71	94.34	8.66	17.05	9.86	5.50	24.64	10.08	40.86	39.87	438.81
Lowest Range	68.33	101.67	31.67	75.25	5.53	6.72	6.65	3.47	12.27	4.75	32.32	27.50	184.36
Highest Range	84.33	123.33	41.00	126.42	13.53	25.36	12.57	12.03	36.30	15.73	46.27	51.90	1205.55
PCV (%)	4.44	3.47	6.47	11.24	18.73	17.63	11.95	26.35	15.13	22.00	6.21	10.96	29.22
GCV (%)	4.27	3.31	5.11	11.09	16.86	16.94	10.33	25.18	14.30	20.92	5.27	10.88	27.95
ECV (%)	1.24	1.03	3.97	1.87	8.17	4.86	6.01	7.77	4.94	7.97	3.28	1.40	8.51
h ² (Broad Sense)	0.92	0.91	0.62	0.97	0.81	0.92	0.75	0.91	0.89	0.88	0.72	0.98	0.92
Genetic Adv. as % of mean	8.44	6.51	8.32	22.53	31.26	33.55	18.40	49.57	27.85	30.70	9.21	22.22	76.01

Where, DF = Day To 50 % Flowering, DM = Day To 75 % Maturity, GFD = Grain Filling Duration, PH = Plant Height, NTPP = Number of Tillers Per Plant, PED. L. = Peduncle Length, SL= Spike Length, AL = Awn Length, BYPP = Biological Yield Per Plant, SYPP = Seed Yield Per Plant, HI = Harvest Index.

due to additive gene action which supports the findings of Virender *et al.* (2015), Pandey *et al.* (2016), Turan *et al.* (2017), Chethana and Rudranaik (2017) and Chandra *et al.* (2019). It is, therefore, such a finding could be effective for direct selection of promising lines for targeted traits.

Categorization of Genotypes based on AUDPC

In the present study, AUDPC was calculated to identify the highly resistance genotypes with better yielding by grouping the genotypes into resistance, moderately resistance, moderately susceptible and susceptible classes.

Based on the genetic makeup of genotypes and severity level, plant breeders/researchers may choose these lines and design the research strategies to improve crop plants for trait interest. Similar results also published by Khan and Chowdhury (2011); Dibya *et al.* (2020); Kumar *et al.* (2020); and Mahapatra *et al.* (2020). Findings also showed that out of 93 wheat genotypes grown in normal condition, 31 genotypes fall into the resistance category having AUDPC ranging from 141.15 to 198.76 of which 10 best genotypes based on lower AUDPC presented in Table 5. Of which Raj 3814 and DBW 166 genotypes scored the lowest AUDPC value of 141.15 followed by FLW 10 (148.35), WR 544 (148.35), Chiriya#3 (155.90), HD 2285 (157.00), HD 3043 (161.32), Yangmai#6 (162.76), Mon/Ald (169.96), FLW 16 (171.40) and DBW 88 (175.52) with satisfactory yield and for other traits. Chandra *et al.* (2019) also published their findings, revealing that most genotypes were moderately resistant and susceptible.

Similarly, in artificial condition, 16 tagged as resistance genotypes having AUDPC ranged from 184.36 to 292.39 of which 10 best genotypes (RAJ (181.36), HI 8737 (218.93), Yangmai#6 (223.25), Ning 8201 (236.21), HD 2733 (236.32), LOK 1(237.65), DBW 168 (246.29), DBW 173 (249.29), Mon/Ald (252.06) and HI 8708 (262.14) scored lowest AUDPC with good yield (Table 6). The moderately resistant category consisted of 40 genotypes, moderately susceptible 27 and 10 susceptible genotypes, respectively. These findings agreed by Kumari *et al.* (2018), Dibya *et al.* (2020), Kumar *et al.* (2020) and Mahapatra *et al.* (2020).

Based on excellent findings, we have noticed that genotypes grown in normal condition differed for their resistance reaction as compared to the genotypes grown in artificial epiphytotic condition which could be because of more pathogenic load in artificially epiphytotic condition than genetic makeup of genotypes too (Elliot *et al.*, 2002) and also consistency temperatures with 100% relative humidity which is favorable for disease development (Duveiller *et al.* (1998). In both the conditions, genotype Raj 3814 exhibited very less disease severity hence, we considered this one as highly resistance genotype by comparing the performance of resistance checks like Ning 8201 and Yangmai#6 genotype, thus such resistance genotypes could be used as donor parent or even could be released as variety (Dhakal *et al.* 2020). Further, it is found that in both conditions, genotypes, namely HD 3171, HD 4730, HUW 468, HUW 55 and Sonalika, expressed severe symptoms and responses to higher degree of disease severity considered as highly susceptible genotypes. For crop improvement point of view, highly resistant and/or highly susceptible genotypes with potential role could be used as donor in hybridization programme for developing superior cultivars/genotypes.

Phenotypic traits such as leaf angle and waxiness indirectly play an important role in identifying the resistance genotypes against resistance to spot blotch disease. Our study evinced that out of 93, 35 genotypes showed

erect leaf angle (37.63%), 28 were semi erect (30.10%), 20 genotypes were semi-drooping (21.50%) and 10 genotypes exhibited drooping pattern (10.75%). Similarly, for leaf waxiness 17 genotypes (18.27%) showed waxiness, 47 genotypes (50.53%) exhibited semi waxy pattern and 29 were non-waxy (30.20%). The frequency distribution of 93 genotypes based on leaf angle and waxiness is presented in Figure 2 (a-b), respectively. All 10 genotypes identified resistance in each environment exhibit either erect or semi-erect leaf stature except HD 2265 HI 8078 genotype. Similarly, most resistance genotypes confer waxy or semi waxy leaf for leaf waxiness except for HD 3043 genotype in normal and HI 77 in artificial conditions. The mechanism behind these phenotypes is that the presence of wax on leaf or stem does not allow water droplets to retain on leaf which could create humidity for spore germination and acts as a barrier for entry of the pathogen. Similarly, erect leaf stature drains out water drops and hinders spore germination. Thus, genotypes having either erect or semi erect leaves and waxy or semi waxy are positively associated with resistance to spot blotch hence, such genotypes could be exploited to breed the promising wheat genotypes (Joshi and Chand, (2002); Prasad *et al.* (2013) and Chandra *et al.* (2019) for human being.

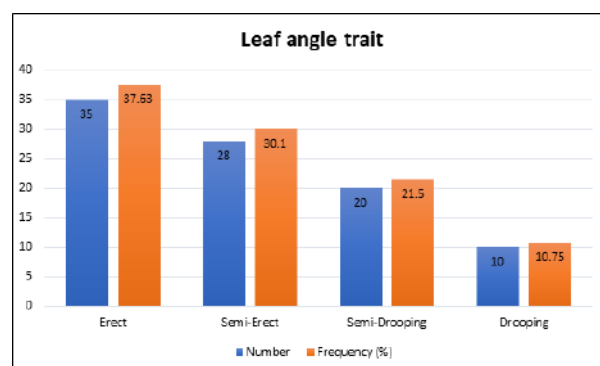


Figure 2a: Graphical representation of 93 wheat genotypes based on their leaf angle

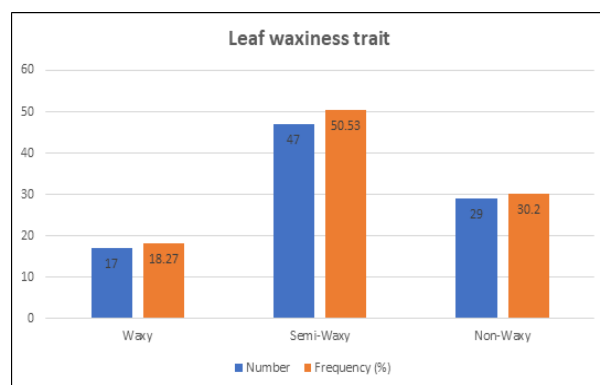


Figure 2b: Graphical representation of 93 wheat genotypes based on their leaf waxiness.

Table 5: Mean performance of best 10 resistant genotypes based on AUDPC in normal condition

S.No.	Genotype	DF	DM	GFD	PH	NTPP	PED L	SL	AL	BYPP	SYPP	HI	1000 GW	AUDPC	Leaf angle	Leaf waxiness
1	DBW 166	77.33	113.33	36.00	90.76	7.80	14.67	9.32	4.30	20.02	11.99	42.91	34.57	141.15	Semi Erect	Semi Waxy
2	RAJ 3814	80.00	116.33	36.33	122.85	10.07	22.59	7.62	6.53	23.18	9.21	39.69	41.77	141.15	Erect	Semi-Waxy
3	FLW 10	77.33	110.33	33.00	74.69	9.33	15.60	9.79	5.04	21.60	10.98	39.54	36.27	148.35	Erect	Waxy
4	WR 544	75.00	116.66	41.66	98.81	8.13	19.79	11.03	5.91	24.20	9.61	39.72	40.76	148.35	Erect	Semi Waxy
5	Chirya#3	72.33	106.67	34.33	94.53	9.73	15.60	9.47	5.84	25.93	11.41	44.02	44.92	155.90	Semi Erect	Semi Waxy
6	HD 2285	69.00	111.00	42.00	83.99	10.00	13.08	10.31	5.94	23.37	9.75	41.70	44.97	157.00	Erect	Non-Waxy
7	HD 3043	77.00	114.67	37.67	96.02	9.73	17.91	9.40	6.97	25.07	9.93	39.58	42.67	161.32	Erect	Semi Waxy
8	Yangmai#6	75.00	112.67	37.67	114.07	11.00	15.66	7.22	5.44	27.63	12.44	45.03	43.93	162.76	Erect	Waxy

Table 6: Mean performance of best 10 resistant genotypes based on AUDPC in diseased inoculated condition

S.No.	Genotype	DF	DM	GFD	PH	NTPP	PED L	SL	AL	BYPP	SYPP	HI	1000 GW	AUDPC	Leaf angle	Leaf waxiness
1	RAJ 3814	81.00	118.00	37.00	126.42	9.20	23.74	7.91	7.04	22.52	11.09	39.34	42.40	184.36	Semi Erect	Semi-Waxy
2	HI 8737 (D)	77.00	115.67	38.67	76.44	8.87	19.07	6.88	10.34	25.57	10.18	39.87	44.37	218.93	Semi Droop	Non-Waxy
3	Yangmai#6	74.67	111.33	36.67	113.13	11.00	14.56	7.10	5.44	27.86	11.74	42.17	41.14	223.25	Erect	Waxy
4	Ning 8201	76.33	111.67	35.33	111.34	11.97	19.00	12.14	5.16	28.26	11.91	41.87	41.44	236.21	Erect	Semi Waxy
5	HD 2733	81.00	115.00	34.00	88.26	8.53	13.90	10.48	4.54	24.57	9.74	39.66	41.43	236.32	Semi Erect	Semi Waxy
6	LOK 1	69.67	104.00	34.33	89.00	7.93	18.37	9.41	6.00	26.52	10.08	38.01	39.00	237.65	Semi Erect	Semi Waxy
7	DBW 168	80.00	115.67	35.67	94.77	7.67	18.16	9.04	6.67	17.96	7.17	39.93	46.57	246.29	Semi Erect	Waxy
8	DBW 173	76.67	114.00	37.33	92.19	6.33	13.78	10.24	4.47	22.50	8.58	38.13	42.00	246.29	Semi Erect	Waxy

Association of Spot Blotch Disease with Yield its Contributing Traits

The correlation results of 13 characters for 93 wheat genotypes grown in normal as well as in artificial epiphytotic conditions are presented in Table 7 and 8. Findings reveals that AUDPC established a negative correlation with important characters like seed yield per plant ($r = -0.33$), days to flowering ($r = -0.27$) and tillers per plant ($r = -0.13$) in normal condition. Similarly, in a diseased condition, it showed negative correlation with seed yield per plant ($r = -0.20$), days to 50% flowering ($r = -0.17$), days to 75% maturity ($r = -0.13$) and plant height ($r = -0.20$). However, seed yield per plant (gm) showed positive correlation with biological yield per plant ($r = 0.89$), number of tillers per plant ($r = 0.55$), harvest index ($r = 0.45$), plant height ($r = 0.15$) and grain filling duration ($r = 0.12$) in normal condition. Whereas, in diseased conditions, it showed positive correlation in highest

magnitude with biological yield per plant ($r = 0.92$), tillers per plant ($r = 0.55$), harvest index ($r = 0.47$) and 1000 grain weight ($r = 0.17$) but negative and significant correlation with AUDPC ($r = -0.20$) and days to 50% flowering ($r = -0.23$) was manifested.

The above findings revealed that in normal conditions, negative correlation of AUDPC with seed yield per plant and other traits depicted that earliness would escapes the disease and intern increases grain yield by reducing disease progress. While the plant height is not common factor to correlate with disease spread as disease will progress from bottom to top of the plant and tall plant type will score less AUDPC. We have identified 10 genotypes expressed resistance reactions evinced tall height as given in Table 5. But earlier report says that increasing the plant height would reduces disease spread (Sharma, *et al.* (2006); Prasad *et al.* (2013); Singh *et al.* (2016) and Turan *et al.* (2017) which could

Table 7: Phenotypic correlation of spot blotch disease with yield and its component traits studied in normal condition

	DF	DM	GFD	PH	SPP	PED L.	SL	AL	BYPP	HI	1000GW	AUDPC	SYPP
DF	1.000	0.796**	-0.103	0.091	-0.116	-0.016	0.153*	-0.069	-0.266**	-0.017	-0.010	-0.178**	-0.230**
DM		1.000	0.517**	0.129*	-0.069	0.054	0.160**	-0.049	-0.103	0.014	-0.011	-0.130*	-0.077
GFD			1.000	0.082	0.052	0.108	0.043	0.0128	0.207**	0.045	-0.004	0.037	0.197**
PH				1.000	0.209**	0.533**	0.185**	-0.060	0.188**	-0.158*	0.231**	-0.202**	0.100
SPP					1.000	0.011	-0.020	0.0875	0.576**	0.111	0.087	0.063	0.550**
PED L.						1.000	0.005	0.241**	-0.038	-0.114	0.063	-0.052	-0.088
SL							1.000	-0.299**	-0.073	0.026	-0.006	-0.094	-0.054
AL								1.000	0.110	-0.123*	0.154**	-0.205**	0.046
BYPP									1.000	0.108	0.209**	0.111	0.925**
HI										1.000	-0.033	0.022	0.473**
1000SW											1.000	-0.023	0.175**
AUDPC												1.000	-0.208**

Table 8: Phenotypic correlation of spot blotch disease, yield and its component traits studied artificial condition

	DF	DM	GFD	PH	SPP	PED L.	SL	AL	BYPP	HI	1000 GW	AUDPC	SYPP
DF	1.000	0.703**	0.306**	0.142*	-0.107	-0.0910	-0.047	-0.129*	-0.222**	-0.037	-0.099	-0.277**	-0.204**
DM		1.000	0.460**	0.079	-0.059	-0.0439	0.001	0.017	-0.076	0.071	0.107	-0.153	-0.101
GFD			1.000	-0.070	0.054	0.0549	0.061	0.185**	0.174**	0.048	0.268**	0.141*	0.120*
PH				1.000	0.263**	0.5159**	0.017**	-0.153*	0.226**	0.082	-0.241**	0.108	0.159**
SPP					1.000	0.1654**	0.101	0.071	0.561**	0.120*	-0.301**	-0.135*	0.5554**
PED L.						1.0000	0.063	0.156**	0.068	-0.123*	-0.025	-0.034	0.001
SL							1.000	-0.192**	0.108	-0.038	-0.157**	-0.071	0.081
AL								1.000	0.095	-0.160**	0.008	-0.175**	0.012
BYPP									1.000	0.022	-0.181**	-0.144*	0.899**
HI										1.000	-0.289**	-0.010	0.452**
1000 GW											1.000	-0.066	-0.290
AUDPC												1.000	-0.332**

*, ** Significant at 5 and 1% levels, respectively.

be better for plant breeders. Seed yield per plant showed strong correlation with biological yield per plant followed by number of tillers per plant and other studied traits in normal conditions which is also supported by Sharma *et al.* (2006) and Kumar *et al.* (2009). Similarly, in a diseased condition, a significant association was observed between seed yield per plant and biological yield per plant followed by the number of effective tillers per plant, harvest index and 1000 grain weight. Based on such findings/estimates, it is concluded that indirect selection of these traits may increase grain yield of the crops by applying strong selection pressure (Virender *et al.* (2015); Singh *et al.* (2016); Chethana *et al.* (2018); Kumari *et al.* (2018) and Chandra *et al.* (2019). Whereas, the negative association of grain yield found with days to 50% flowering and days to maturity, indicating completion of generation very early and may produce high grain yield in bread wheat, similar reports published by Chethana *et al.* (2018) and Chandra *et al.* (2019). Grain filling duration positively correlated with AUDPC as it is the period between spike emergence and maturity which is critical for spot blotch disease development. One of the excellent wheat genotype namely HUW 234 is early in flowering but it also exhibit maximum spot blotch disease severity since it coincides with disease occurrence because of long grain filling duration and also has actual yield recovery property under diseased conditions.

Summary and Conclusion

Since spot blotch disease of wheat caused *B. sorokiniana* pathogen is one of the very destructive disease and there is the unavailability of enough resistance genotypes along with better yielding so, a set of 93 diverse wheat genotypes (most of them are released) are evaluated at the Agriculture Research Farm (known as hot spot for spot blotch), Institute of Agricultural Sciences, Banaras Hindu University in normal as well as artificial epiphytotic conditions in order to identify the resistance and high yielding donors to be used for crop improvement. Present findings reveal a considerable variation for spot blotch disease resistance and other traits among 93 genotypes studied in normal and diseased conditions. Identified genotypes with potential grain yield are recommended for its use in resistance breeding programme to breed the desirable genotypes, saving the yield loss through an ecofriendly approach and enhancing the productivity of wheat crops to supply the demand of rapidly growing population. In spite of this, majority of genotypes that showed either resistance or moderately resistance reactions and having erect/semi-erect leaves as well as appearance of wax on plants, may be considered as strong morphological marker to select the resistance genotypes and incorporate genes for such traits into targeted genotypes during a breeding programme for crop improvement.

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RESEARCH ARTICLE

Assessment of Genetic Diversity in Finger Millet [*Eleusine coracana* (L.) Gaertn.] Genotypes

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Abstract

The experimental material comprised 148 diverse finger millet genotypes representing collections from all over India. All the genotypes were characterized for 19 grain yield and yield-related traits. They were evaluated to assess diversity among various genotypes at the Agricultural Research Station, Vizianagaram during *khari*f, 2019–20. Significant variations were observed for all the traits studied. Analysis of D² statistics grouped them into 22 clusters. Cluster I was the largest group with the maximum number of genotypes (103), followed by cluster XI and XIV with 13 and 11 genotypes, respectively, while 18 were solitary clusters. The inter-cluster distance was highest between clusters XVII and XXII, followed by clusters XIII and XXII. Among the 19 quantitative traits studied, the most important trait contributing to the divergence was ear length, followed by days to 50% flowering and grain yield. Based on mean values and inter-cluster distances, IC0329452 × IC0474832, IC043734 × IC0474832, IC0475882 × IC0474832, IC0474832 × IC028353 and IC0474832 × IC0478862 crosses are proposed to attain multiple desirable characters in a single genotype.

Keywords: Cluster, D² statistic, Finger millet, Genetic diversity.

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Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.) is the most important small millet crop. It is cultivated in more than 25 countries in Africa and Asia. India is a major producer of finger millet in Asia, occupying an area of 1193.70 thousand hectares with a production of 1982.90 thousand tonnes and productivity of 1661.00 kg per hectare (Anonymous, 2014). It is largely grown in the southern states of India. Finger millet gained its name as “Nutri-cereal” since the grains are nutritionally superior to many cereals in terms of protein (7–10%), calcium (344 mg/100 g), iron (3–4 mg/100 g) and other mineral content. It is also rich in phosphorus (283 mg/100 g) and potassium (408 mg/100 g). Moreover, carbohydrates in finger millet have the unique property of slow digestibility and are a good diet for diabetics. Finger millet is a drought-tolerant crop that can thrive with little water. It can also withstand high temperatures and thrive in poor, deteriorated soils. It may be grown in varied climates (Anuradha *et al.*, 2021).

Despite having many health benefits, finger millet cultivation has been reduced and replaced by other commercial crops over the years. Now it is again gaining importance as people are becoming increasingly health-conscious. A decrease in the area can only be compensated for by increased productivity. Hence, finger millet improvement should be mainly focused on developing high-yielding varieties. The productivity of finger millet is also affected by a few diseases like a blast and banded blight, which are likely to

cause more than 50% yield losses (Patro *et al.*, 2020). Though the ultimate aim of a breeder is to develop a high-yielding variety, the sustainability of a variety for a longer period in a farmer's field depends on its disease resistance to major diseases. As a result, developing a high-yielding variety with disease resistance is critical in the advancement of new finger millet cultivars. This can be achieved by exploiting the agrobiodiversity of finger millet lines for selecting genotypes with *per se* high yield and disease resistance or else for selecting genetically diverse parents with desirable characters to make crosses for creating variability. In this context, Mahalanobis D² statistics is an age-old effective tool utilized in quantifying the degree of divergence at a genetic level and also provides a quantitative measure of association between geographic and genetic diversity based on generalized distance (Mahalanobis, 1936). Genetic divergence is a tool to select diverse parents for utilization in specific hybridization to generate greater variability in self-pollinated crops. This in turn, provides scope for the breeder to select high-yielding varieties along with desirable traits. Hence, the present study was carried out to determine the nature and magnitude of genetic divergence among 148 finger millet genotypes under the Consortia Research Platform (CRP) agro biodiversity project.

Materials and Methods

The experimental material was comprised of 148 finger millet genotypes, including three checks, MR 6, GPU 67, and VL 352. They were obtained from the ICAR-Indian Institute of Millet Research, Hyderabad, which included diverse germplasm collected from different parts of the country (Table 1). All these genotypes were sown on 31.07.2019 at the Agricultural Research Station, Vizianagaram, Andhra Pradesh, located at latitude: 18.12' N, longitude: 83.40' E and an altitude of 63 m. MSL, comprising red sandy loam soil. The weather data for the location was presented in Figure 1. The layout was a completely randomized block design (RCBD) with two replications and a spacing of 30 cm between rows and 10 cm between plants. Each genotype was sown in two rows of 3 m in length. Fertilizers, DAP (87 kg/ha), MOP

(42 kg/ha) and urea (22 kg/ha) were applied basally at the time of land preparation and the remaining 22 kg/ha of urea was applied three weeks after sowing. Standard management practices were followed, except for disease management. Observations were recorded on five plants and means were computed for the traits viz., plant height (cm), number of productive tillers/plant, number of fingers/ear, ear length (cm), finger length (cm), finger width (cm), number of leaves/main tiller, flag leaf length (cm), flag leaf width (cm), length from top node to leaf sheath junction (cm), and peduncle length (cm). Days to 50% flowering and days to maturity were recorded by visualizing the entire plot. Fodder and grain yield were recorded per plot basis and then converted to per hectare. Disease data was recorded under natural field conditions. Leaf blast (Table 2) was recorded by using 0–5 scale and the percent disease index (PDI) was calculated by using the following formula:

$$\text{PDI} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings}} \times 100$$

$$\text{Neck blast (\%)} = \frac{\text{No. of infected panicles}}{\text{Total no. of panicles}} \times 100$$

$$\text{Finger blast (\%)} = \frac{\text{No. of infected fingers}}{\text{Average number of fingers}} \times 100$$

$$\text{Banded blight (\%)} = \frac{\text{No. of infected plants}}{\text{Total number of plants}} \times 100$$

Mahalanobis D² statistic was used to assess the diversity among genotypes and were grouped into clusters using Tocher's method described by Rao (1952).

Results and Discussion

The variance analysis revealed significant differences among 148 genotypes for all the 19 characters included under study (Table 3), indicating that the experimental material consisted of more variation for all the traits. Similar results were obtained by Kumar *et al.*, (2010), Karad and Patil., (2013) and Anuradha *et al.*, (2020).

The quantitative assessment of genetic divergence was obtained by adopting the Mahalanobis D² statistic for yield and its contributing characteristics. These 148 genotypes were grouped into 22 clusters (Tables 3 and 4). Among various clusters, cluster I contained a maximum of 103 genotypes, followed by cluster XI consisting of 13 genotypes, cluster XIV with 11 genotypes, and cluster XIX with three genotypes, while the remaining clusters had a solitary genotype. It indicated that few genotypes were very diverse from others, contributing much to genetic diversity. Traits that contributed most to this diversity in these genotypes were discussed later. Two checks, viz., MR 6 and VL 352, were grouped into the largest cluster I, and the remaining check, GPU 67, was grouped into the second largest cluster XI. It implies that the checks used in the study were also diverse. Similar results were reported earlier by Anantharaju and Meenakshiganesan (2008), Kumar *et al.*, (2010), Karad

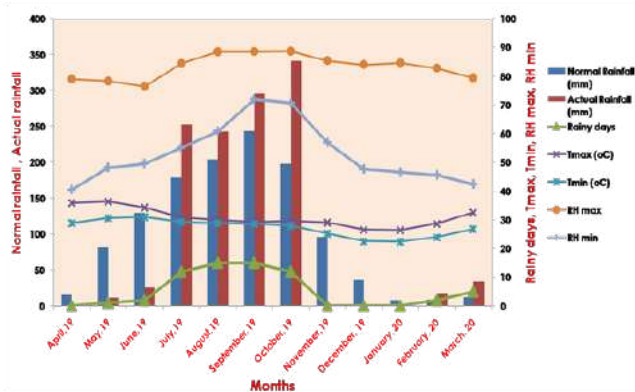


Figure 1: Weather data at the experimental site during kharif, 2019.

Table 1: List of 148 finger millet genotypes and their origin.

S. No.	Genotype ID	Accession Number	IC/EC Number	Country of Origin	State
1	FIN 2853	GE 20	IC0476259	India	Unknown
2	FIN 2872	GE 188	IC0476362	India	Unknown
3	FIN 2891	GE 198	IC0476439	India	Unknown
4	FIN 2899	GE 108	IC0476484	India	Unknown
5	FIN 2901	GE 61	IC0476492	India	Unknown
6	FIN 2936	GE 162	IC0476623	India	Unknown
7	FIN 2960	GE 127	IC0476742	India	Bihar
8	FIN 2998	GE 10	IC0477078	India	Unknown
9	FIN 3003	GE 2	IC0477289	India	Unknown
10	FIN 3004	GE 125	IC0477419	India	Bihar
11	FIN 3007	GE 396	IC0475528	India	Tamil Nadu
12	FIN 3017	GE 276	IC0475790	India	Unknown
13	FIN 3022	GE 345	IC0475848	India	Unknown
14	FIN 3026	GE 363	IC0475937	India	Unknown
15	FIN 3062	GE 372	IC0476193	India	Unknown
16	FIN 3068	GE 374	IC0476246	India	Unknown
17	FIN 3086	GE 313	IC0476326	India	Unknown
18	FIN 3088	GE 280	IC0476333	India	Unknown
19	FIN 3090	GE 267	IC0476345	India	Unknown
20	FIN 3296	GE 527	IC0476587	India	Unknown
21	FIN 3318	GE 545	IC0475654	India	Unknown
22	FIN 3321	GE 676	IC0475716	India	Unknown
23	FIN 3336	GE 554	IC0475868	India	Unknown
24	FIN 3376	GE 679	IC0476124	India	Unknown
25	FIN 3422	GE 586	IC0476381	India	Unknown
26	FIN 3473	GE 724	IC0475746	India	Uttarakhand
27	FIN 3496	GE 776	IC0475802	India	Unknown
28	FIN 3525	GE 769	IC0476020	India	Unknown
29	FIN 3532	GE 782	IC0476095	India	Unknown
30	FIN 3538	GE 750	IC0476138	India	Unknown
31	FIN 3583	GE 861	IC0475407	India	Unknown
32	FIN 3608	GE 888	IC0475653	India	Unknown
33	FIN 3622	GE 916	IC0475759	India	Unknown
34	FIN 3639	GE 889	IC0475858	India	Unknown
35	FIN 3689	GE 866	IC0476213	India	Unknown
36	FIN 3726	GE 1059	IC0475382	India	Unknown
37	FIN 3765	GE 1007	IC0475697	India	Unknown
38	FIN 3321	GE 676	IC0475716	India	Unknown
39	FIN 3782	GE 1084	IC0475780	India	Unknown
40	FIN 3789	GE 961	IC0475814	India	Unknown
41	FIN 3793	GE 1022	IC0475826	India	Unknown
42	FIN 3847	GE 1093	IC0475246	India	Unknown
43	FIN 3848	GE 1199	IC0475251	India	Unknown
44	FIN 3855	GE 1185	IC0475344	India	Unknown

45	FIN 3856	GE 1117	IC0475355	India	Unknown
46	FIN 3871	GE 1131	IC0475416	India	Unknown
47	FIN 3883	GE 1167	IC0475477	India	Unknown
48	FIN 3901	GE 1197	IC0475620	India	Unknown
49	FIN 3928	GE 1174	IC0475882	India	Unknown
50	FIN 3952	GE 1175	IC0476459	India	Unknown
51	FIN 4203	GE 1551	IC0475019	India	Unknown
52	FIN 4213	GE 1564	IC0475073	India	Unknown
53	FIN 4219	GE 1557	IC0475125	India	Unknown
54	FIN 4260	GE 1528	IC0475436	India	Unknown
55	FIN 4288	GE 1671	IC0474816	India	Unknown
56	FIN 4303	GE 1666	IC0474956	India	Unknown
57	FIN 4331	GE 1603	IC0475164	India	Unknown
58	FIN 4336	GE 1584	IC0475201	India	Unknown
59	FIN 4349	GE 1638	IC0475270	India	Unknown
60	FIN 4462	GE 1780	IC0475095	India	Unknown
61	FIN 4482	GE 1741	IC0475244	India	Unknown
62	FIN 4483	GE 1773	IC0475252	India	Unknown
63	FIN 4491	GE 1795	IC0475374	India	Unknown
64	FIN 4493	GE 1712	IC0475386	India	Unknown
65	FIN 4498	GE 1723	IC0475473	India	Unknown
66	FIN 4512	GE 1869	IC0474775	India	Uttarakhand
67	FIN 4516	GE 1902	IC0474782	India	Unknown
68	FIN 4524	GE 1935	IC0474806	India	Unknown
69	FIN 4527	GE 1853	IC0474820	India	Unknown
70	FIN 4533	GE 1818	IC0474832	India	Unknown
71	FIN 4536	GE 1829	IC0474840	India	Unknown
72	FIN 4542	GE 1850	IC0474862	India	Unknown
73	FIN 4578	GE 1910	IC0474970	India	Unknown
74	FIN 4602	GE 1924	IC0475100	India	Unknown
75	FIN 4644	113/84-16	IC0478942	India	Unknown
76	FIN 4794	IE-2431	IC0473590	India	
77	FIN 4830	IE-2490	IC0473636	India	Unknown
78	FIN 4863	IE-2598	IC0473714	India	Unknown
79	FIN 4866	IE-2601	IC0473717	India	Unknown
80	FIN 4874	IE-2612	IC0473726	India	Unknown
81	FIN 4881	IE-2621	IC0473734	India	Unknown
82	FIN 4957	IE-2790	IC0473889	India	
83	FIN 4958	IE-2791	IC0473890	India	Unknown
84	FIN 4961	IE-2812	IC0473909	India	Unknown
85	FIN 5088	IE-3070	IC0474128	India	Unknown
86	FIN 5154	IE-3150(7-74-1)	IC0474199	India	Unknown
87	FIN 5158	IE-3154(10-5-4-1)	IC0474203	India	Unknown
88	FIN 5160	IE-3143-1	IC0479215	India	Unknown
89	FIN 5200	IE-3250	IC0474297	India	Unknown
90	FIN 5301	NC-57078	IC0007952	India	Unknown

91	FIN 5337	5/81 - 7	IC0049936	India	Tamil Nadu
92	FIN 5341	13/81 - 2	IC0049942	India	Karnataka
93	FIN 5406	96/81 - 2	IC0050000	India	Karnataka
94	FIN 5412	170-B	IC0071333	India	Kerala
95	FIN 5413	21/83-22175	IC0071338	India	Kerala
96	FIN 5425	A-1128	IC0087508	India	Sikkim
97	FIN 5433	C-2009450	IC0087540	India	Jharkhand
98	FIN 5453	GE-925	IC0478720	India	Unknown
99	FIN 5458	EKR- 180	IC0478760	India	Unknown
100	FIN 5460	GE-1019	IC0478764	India	Unknown
101	FIN 5461	EKEP- 10	IC0478776	India	Unknown
102	FIN 5462	GE-872	IC0478780	India	Unknown
103	FIN 5464	GE-629	IC0478790	India	Unknown
104	FIN 5514	GE-3196	IC0478792	India	Unknown
105	FIN 5540	GE-3311	IC0478444	India	Unknown
106	FIN 5562	GE-3372	IC0478809	India	Unknown
107	FIN 5578	GE-3548	IC0478442	India	Unknown
108	FIN 5596	GE-3990	IC0478656	India	Unknown
109	FIN 5599	GE-4073	IC0478693	India	Unknown
110	FIN 5601	GE-3736	IC0478710	India	Unknown
111	FIN 5601	GE-3736	IC0478710	India	Unknown
112	FIN 5620	GE-3839	IC0478883	India	Unknown
113	FIN 5668	GE-4492	IC0478862	India	Unknown
114	FIN 5717		IC0096774	India	Maharashtra
115	FIN 5731		IC0096815	India	Maharashtra
116	FIN 5732		IC0096817	India	Maharashtra
117	FIN 5735		IC0096827	India	Maharashtra
118	FIN 5736		IC0096844	India	Maharashtra
119	FIN 5849	96/82-25	IC0069594	India	Kerala
120	FIN 5893	K-2838TCR-425	IC0087530	India	Tamil Nadu
121	FIN 5960	GE-4736IE-2361	IC0473528	India	Unknown
122	FIN 5994	GE-4772IE-2410	IC0473571	India	Unknown
123	FIN 6077	GE-4867IE-2753	IC0473855	India	Unknown
124	FIN 6176	GE-4966IE-2958	IC0474034	India	Unknown
125	FIN 6188	GE-4979IE-3164	IC0474213	India	Unknown
126	FIN 6269	GE-5065IE-3333	IC0474370	India	Unknown
127	FIN 6347	M-112/81-6TCR-148	IC0069583	India	Karnataka
128	FIN 6364	M-132/84-8TCR-248-A (NC-71411X)	IC0071411-A	India	Unknown
129	FIN 6423	VRS-MF-872	IC0261985	India	Uttarakhand
130	FIN 6478	NSS-7890	IC0283815	India	Andhra Pradesh
131	FIN 6493	NSS-7928	IC0283853	India	Andhra Pradesh
132	FIN 6525	VR-MF-1371	IC0281756	India	Uttarakhand
133	FIN 6526	VR-MF-1373	IC0281758	India	Uttarakhand
134	FIN 6530	VR-MF-1383	IC0281768	India	Uttarakhand
135	FIN 6546	KVC-56	IC0329452	India	Himachal Pradesh

136	FIN 6558	JBT27/76	IC0331685	India	Jharkhand
137	FIN 6560	JBT27/94	IC0331687	India	Jharkhand
138	FIN 6563	JBT27/104	IC0331690	India	Jharkhand
139	FIN 6584	BS-9111	IC0308922	India	Andhra Pradesh
140	FIN 6585	BS-9143	IC0308929	India	Andhra Pradesh
141	FIN 6624	LS-47/2001	IC0344178	India	Maharashtra
142	FIN 6658	LS-41/2001	IC0344172	India	Maharashtra
143	FIN 6723	BAS-145	IC0345081	India	Andhra Pradesh
144	FIN 6754	VKS/SCC-8/13	IC0347252	India	Bihar
145	FIN 6772	DPRR-77	IC0361119	India	Uttarakhand
146	GPU 67			India	Karnataka
147	MR 6			India	Karnataka
148	VL 352			India	Uttarakhand

Table 2: Standard evaluation system (SES) scale for leaf blast disease.

Score	Description	Reaction
0	No lesions/symptoms on leaves	No disease/HR
1	Small brown specks of pinhead to slightly elongate, necrotic grey spots with a brown margin, less than 1% area affected	R
2	A typical blast lesion elliptical, 5–10 mm long, 1–5% of leaf area affected	MR
3	A typical blast region elliptical, 1–2 cm long, 6–25 % of leaf area affected	MS
4	26–50% leaf area affected	S
5	More than 50% of leaf area affected with coalescing lesions	HS

and Patil, (2013) and Anuradha *et al.*, (2017). The origin of many genotypes was unknown (Table 1). However, it was observed that genotypes collected from Bihar, Tamil Nadu, Uttarakhand, Kerala, Jharkhand, Maharashtra, and Andhra Pradesh were grouped into different clusters irrespective of their origin. There was no parallelism between the origin of genotypes and grouping patterns. This is in accordance with the results of Shinde *et al.*, (2013).

Intra and inter-cluster distances were worked out using D^2 values from divergence analysis (Table 5, Figure 2). The inter-cluster distance values ranged widely, with a minimum value of 10.39 between clusters IV and X and a maximum value of 108.13 between clusters XVII and XXII, indicating high diversity between the genotypes of XVII and XXII clusters. It was followed by clusters XIII and XXII (91.36) and XVII and XX (88.29). This suggested that there was wide genetic diversity between these clusters. The maximum heterosis is expected from the crosses with parents of these clusters. Cluster XXII, having a solitary genotype, was found to have a higher inter-cluster distance with almost 10 clusters (XVII, XIII, IX, XX, XV, XVI, IV, XXI, XXI, XVIII) recording more than 70 inter-cluster distance. The least inter-cluster distance with this cluster was observed with cluster VIII, which was also a solitary cluster. But even this inter-cluster distance was higher than the remaining 92 inter-cluster distances, implying that the genotypes from this cluster were highly diverse from the genotypes of remaining clusters. The

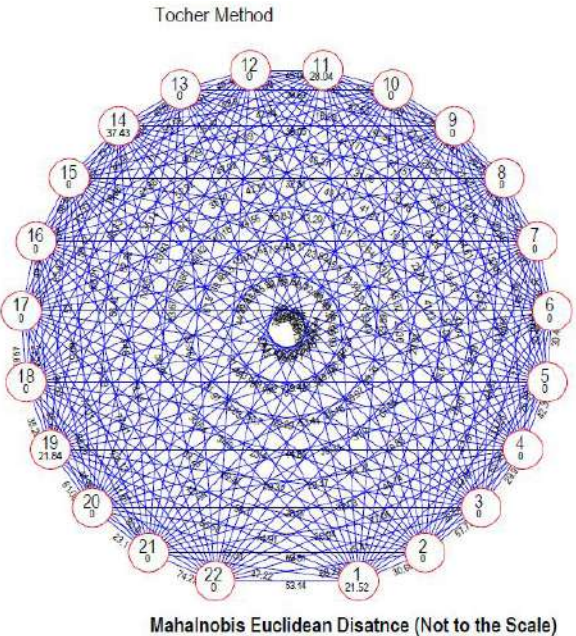


Figure 2: Intra and Inter cluster distance between 22 finger millet clusters

cluster with the maximum number of genotypes recorded lesser inter-cluster distances, with the remaining other clusters indicating a low level of divergence. The intra-cluster distance ranged from 0 (solitary clusters) to 37.43 (XIV). Cluster XIV (37.43), followed by cluster XI (28.04), cluster XIX (21.84) and cluster I (21.52), recorded the next higher intra-

Table 3: ANOVA of 148 finger millet genotypes

Source of Variations	df	Mean Squares													
		DFF	DM	PH	NPT	NFE	EL	FL	FW	PDL	NUL	LN	FLL	FLW	GY
Treatments	147	67.94**	85.32**	82.34**	0.49**	2.38**	7.60**	7.55**	0.02**	6.27**	5.34**	14.08**	39.15**	0.02*	1910371**
Replications	1	0.08	0.57	285.29	2.31	21.19	12.50	4.31	0.00	2.36	0.15	32.49	33.63	0.00	176217
Error	295	7.92	15.86	21.68	0.29	1.02	0.85	0.94	0.00	1.73	3.24	3.95	15.37	0.02	366225

Note: DFF: Days to 50% flowering; DM: Days to maturity; PH: Plant height (cm); NPT: No. of productive tillers per plant; NFE: No. of finger per ear; EL: Ear length (cm); FL: Flag length (cm); FLL: Flag leaf length (cm); FLW: Flag leaf width (cm); GY: Grain yield (q/ha); FY: Fodder yield (q/ha); Peduncle length (cm); NUL: Length from the top node to leaf sheath junction (cm); LN: No. of leaves/main tiller; FLL: Flag leaf length (cm); FLW: Flag leaf width (cm); FY: Fodder yield (q/ha); LB: Leaf blast (Grade); FB: Finger blast (%) and BB: Banded blight (%)

Table 4: Distribution of 148 genotypes of Finger millet into 22 different clusters on the basis of Mahalanobis D² Statistics

Cluster	No. of genotypes	Genotypes
I	103	GE-872 (IC0478780), FIN 5735 (IC0096827), EKEP- 10 (IC0478776), GE 125 (IC0477419), 96/82-25 (IC0069594), GE 1557 (IC0475125), GE 769 (IC0476020), GE 1666 (IC0474956), GE 280 (IC0476333), IE-2431 (IC0473590), GE 1850 (IC0474862), NC-57078(NC-57078), GE 1528 (IC0475436), IE-3070 (IC0474128), GE 1185 (IC0475344), GE 372 (IC0476193), GE 676 (IC0475716), GE 586 (IC0476381), FIN 5732 (IC0096817), VR-MF-1371 (IC0281756), GE 961 (IC0475814), GE 1723 (IC0475473), GE 1869 (IC0474775), BS-9143 (IC0308929), MR 6, 13/81 – 2 (IC0049942), VR-MF-1383 (IC0281768), GE-3736 (IC0478710), GE 1197 (IC0475620), GE-3372 (IC0478809), GE 527 (IC0476587), GE-629 (IC0478790), GE 861 (IC0475407), GE 267 (IC0476345), GE 363 (IC0475937), GE 125 (IC0477419), GE-4979IE-3164 (IC0474213), M-112/81-6TCR-148 (IC0069583), A-1128 (IC0087508), 5/81 – 7 (IC0049936), GE 1910 (IC0474970), BS-9111 (IC0308922), JBT27/76 (IC0331685), VL 352, EKR- 180 (IC0478760), GE 679 (IC0476124), GE 545 (IC0475654), GE-4772IE-2410 (IC0473571), GE 1175 (IC0476459), GE-3839 (IC0478883), JBT27/94 (IC0331687), LS-41/2001 (IC0344172), GE 554 (IC0475868), GE 1584 (IC0475201), GE 374 (IC0476246), GE 1935 (IC0474806), GE 1131 (IC0475416), GE 1059 (IC0475382), GE 724 (IC0475746), GE 198 (IC0476439), GE 1638 (IC0475270), GE 866 (IC0476213), GE-5065IE-3333 (IC0474370), GE-3311 (IC0478444), 96/81–2 (IC0050000), GE 1780 (IC0475095), VKS/SCC-8/13 (IC0347252), GE-4867IE-2753 (IC0473855), GE 1773 (IC0475252), 113/84-16 (IC0478942), GE 1671 (IC0474816), GE1117 (IC0475355), GE 1084 (IC0475780), GE 2 (IC0477289), GE 1022 (IC0475826), GE 1167 (IC0475477),GE 1795 (IC0475374), M-132/84-8TCR-248-A(NC-7141X) (IC0071411-A), GE-1019 (IC0478764), BAS-145 (IC0345081), GE 1093 (IC0475246), IC0096844, IC0096774, IC0096815, GE-3548 (IC0478442), GE 1924 (IC0475100), GE-3990 (IC0478656), GE-4736IE-2361 (IC0473528), GE 782 (IC0476095), DPRR-77 (IC0361119), 6772, 21/83-22175 (IC0071338), IE-2601 (IC0473717), IE-2612 (IC0473726), IE-3143-1 (IC0479215), GE 345 (IC0475848), IE-2812 (IC0473909), GE 1199 (IC0475251), GE 1829 (IC0474840), GE 1741 (IC0475244), GE 127 (IC0476742), IE-2490 (IC0473636), IE-2598 (IC0473714), GE 1007 (IC0475697)
II	1	GE1174 (IC0475882)
III	1	GE 1853 (IC0474820)
IV	1	GE 776 (IC0475802)
V	1	GE 313 (IC0476326)
VI	1	C-2009450 (IC0087540)
VII	1	GE 10 (IC0477078)
VIII	1	K-2838TCR-425 (IC0087530)
IX	1	KVC-56 (IC0329452)
X	1	GE-925 (IC0478720)
XI	13	IE-2790 (IC0473889), VR-MF-1373(IC0281758), IE-2791 (IC0473890), GE 750 (IC0476138), GE 916 (IC0475759), IE-3150(7-74-1) (IC0474199), GE 889 (IC0475858), GE 162 (IC0476623), LS-47/2001 (IC0344178), NSS-7890 (IC0283815), 170-B (IC0071333), GPU 67, GE 1902 (IC0474782)
XII	1	GE 20 (IC0476259)

XIII	1	N55-7928 (IC0283853)
XIV	11	GE 888 (IC0475653), GE 1551 (IC0475019), JBT27/104 (IC0331690), VRS-MF-872 (IC0261985), GE 1564 (IC0475073), GE-3196 (IC0478792), GE 188 (IC0476362), GE-3736 (IC0478710), IE-3250 (IC0474297), GE 61 (IC04764920), GE-4073 (IC0478693)
XV	1	IE-3154(10-5-4-1) (IC0474203)
XVI	1	GE 396 (IC0475528)
XVII	1	GE-4492 (IC0478862)
XVIII	1	GE 276 (IC0475790)
XIX	3	GE 1603 (IC0475164), GE 1712 (IC0475386), GE 108 (IC0476484)
XX	1	GE-4966IE-2958 (IC0474034)
XXI	1	IE-2621 (IC0473734)
XXII	1	GE 1818 (IC0474832)

Table 5: Intra and inter-cluster D2 values among 22 clusters with 148 finger millet [*Eleusine coracana* (L.) Gaertn.] genotypes

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII
I	21.52	30.68	28.01	28.54	32.97	27.90	29.01	29.60	29.83	29.69	36.99	31.89	28.43	31.88	51.91	36.64	33.25	42.27	56.58	53.33	47.22	53.14
II		0	57.78	29.24	65.28	33.26	44.83	37.62	30.15	23.36	39.49	55.62	40.40	41.70	23.96	44.03	34.9	62.37	58.10	74.91	69.51	68.22
III			0	29.95	19.06	26.87	27.10	33.31	43.04	41.13	45.02	33.78	41.96	35.67	77.46	49.66	50.70	23.40	59.32	28.07	26.94	45.41
IV				0	42.37	14.01	20.44	18.6	12.63	10.39	22.74	53.04	28.34	43.88	33.05	49.58	32.15	29.29	44.85	29.17	32.03	77.05
V					0	30.48	19.01	36.24	52.47	44.61	44.26	10.85	52.64	45.50	72.46	43.51	62.41	19.42	53.44	39.38	51.22	40.74
VI						0	24.16	12.90	26.68	17.79	23.83	33.46	41.91	41.31	23.82	48.3	49.86	32.29	43.58	28.16	31.9	49.89
VII							0	31.71	35.25	27.87	29.32	27.15	31.06	49.17	53.29	48.17	38.52	24.33	58.46	39.07	50.97	67.65
VIII								0	26.49	19.41	23.37	40.98	41.77	40.37	32.31	55.83	57.95	44.86	50.29	20.63	26.24	39.33
IX									0	14.29	36.69	57.55	16.23	38.05	50.13	42.51	24.55	44.16	55.88	59.3	43.52	84.57
X										0	30.44	46.32	29.63	42.94	32.69	47.02	35.57	40.18	45.15	46.81	47.59	60.44
XI											28.04	45.09	47.56	52.80	35.87	50.29	52.92	44.50	48.42	39.48	42.91	73.56
XII												0	49.16	47.91	59.58	31.00	61.88	36.14	53.93	60.68	61.5	44.56
XIII													0	40.61	73.04	49.97	18.86	58.83	82.98	74.05	53.67	91.36
XIV														37.43	73.77	46.82	45.35	58.12	68.21	66.42	54.8	56.28
XV															0	60.42	76.66	65.42	52.00	60.57	72.79	78.39
XVI																0	49.21	42.13	27.24	84.53	74.31	77.91
XVII																	0	49.65	72.44	88.29	60.26	108.13
XVIII																		0	35.29	38.45	44.88	71.67
XIX																			21.84	61.06	65.95	80.60
XX																			0	23.10	54.25	
XXI																				0	74.27	
XXII																					0	

Table 6: Mean values of 22 clusters estimated by Tocher's method from 148 finger millet [*Eleusine coracana* (L.) Gaertn.] genotypes

Cluster	GY	DFF	DM	PH	NPT	NFE	EL	FL	FW	LNM	FLL	FLW	NUL	PDL	FY	LB	NB	FB	BB
I	3705.7	69.2	102.1	69.6	3.4	7.2	8.1	6.4	0.9	14.1	40.0	1.2	9.7	9.4	7952.0	2.1	19.6	26.9	23.5
II	5250.0	84.0	116.0	70.7	3.4	7.4	7.5	6.2	1.0	15.4	43.2	1.1	11.4	10.2	7888.8	1.1	12.0	17.4	18.0
III	3105.6	56.0	91.0	71.6	3.3	7.4	9.9	7.5	0.9	13.1	34.8	1.1	12.7	8.8	5944.4	2.2	21.5	29.9	25.3
IV	3511.1	73.0	107.5	68.6	3.4	7.3	12.0	10.1	0.9	12.4	41.4	1.2	10.7	11.9	5166.6	2.5	20.6	28.8	25.2
V	1222.2	59.5	90.0	71.7	3.5	7.7	9.0	8.0	0.8	15.5	39.4	1.0	9.7	8.7	8444.4	3.1	25.3	33.8	27.0
VI	3188.9	71.5	106.0	67.6	2.8	7.9	11.8	9.6	1.0	14.2	34.8	1.0	7.2	10.4	7888.8	1.8	22.7	30.4	25.8
VII	1838.9	67.5	104.0	64.6	3.3	8.7	9.8	8.7	0.8	11.8	48.0	1.2	9.8	9.0	6555.5	2.9	24.9	34.5	27.4
VIII	4344.5	70.0	104.5	65.5	3.2	7.4	12.1	11.5	0.8	17.4	36.2	1.2	10.2	9.9	9833.2	1.5	17.8	24.4	23.1
IX	4994.4	72.5	108.5	69.2	3.4	5.5	9.7	8.8	0.8	12.9	42.4	1.2	7.1	12.5	5555.5	1.2	15.3	21.7	20.5
X	3694.5	76.0	111.0	68.9	2.5	7.3	10.2	8.7	0.8	15.2	41.1	1.2	10.0	12.7	6833.3	1.9	17.6	27.3	24.7
XI	3403.4	74.5	108.5	70.1	3.6	7.7	11.9	10.4	0.9	13.2	42.3	1.2	9.9	9.5	9358.9	2.4	20.4	28.1	23.9
XII	1416.7	66.5	97.0	73.5	2.7	6.7	7.9	6.6	0.8	15.6	37.9	1.1	7.9	5.8	9888.8	2.9	24.3	33.3	26.2
XIII	4350.0	70.0	104.5	61.0	2.5	5.8	8.0	6.0	0.6	9.4	43.1	1.3	8.1	10.8	6333.3	1.3	16.9	22.7	21.4
XIV	4791.9	66.7	98.2	70.1	3.7	7.1	7.7	6.1	0.9	13.8	38.7	1.1	9.6	10.6	9131.2	1.3	16.1	22.8	20.5
XV	3538.9	88.0	121.5	72.9	2.9	7.9	11.9	10.4	1.0	18.4	37.2	1.1	9.1	8.3	8166.6	2.3	20.3	25.8	23.0
XVI	3416.7	72.5	107.5	95.6	3.5	6.8	7.7	6.5	0.8	12.5	41.3	1.0	8.7	6.8	8777.7	2.7	20.9	29.4	24.1
XVII	4144.5	71.5	105.5	71.8	2.6	5.9	6.5	4.4	1.0	9.1	53.4	1.5	8.9	12.9	6944.4	1.6	18.0	24.9	23.1
XVIII	1711.1	61.0	97.5	85.8	3.2	6.7	10.7	8.8	1.0	15.8	47.5	1.1	12.0	9.6	5888.8	2.8	25.0	32.5	26.8
XIX	3122.2	73.2	106.7	100.8	3.2	8.5	11.7	9.9	1.0	14.9	42.9	1.1	10.4	9.8	9481.4	2.7	21.1	29.2	24.7
XX	3044.5	61.5	90.5	66.6	2.9	8.8	15.2	13.6	0.9	15.5	37.4	1.1	13.8	9.0	7722.2	2.8	23.5	31.2	26.0
XXI	4833.3	59.0	93.5	65.6	2.4	5.3	13.6	11.3	1.1	11.9	38.8	1.2	9.9	9.7	8499.9	1.8	14.1	19.6	21.2
XXII	4083.3	61.0	89.5	68.3	2.7	10.3	8.5	7.6	0.8	25.6	35.8	1.0	10.2	7.9	10499.9	1.5	18.0	25.3	22.2

cluster distances, while it was very low for cluster I, along with a lower inter-cluster distance, indicating its less usage in breeding. As usual, inter-cluster distances were higher than the intra-cluster distances, indicating the presence of wider genetic diversity between the clusters rather than within the clusters. Similar results were obtained by Wolie and Belete (2013) and Devaliya *et al.*, (2017).

Cluster means indicate the average performance of all the genotypes present in a particular cluster. The cluster mean values for 19 characters are presented in Table 6. A large gap of 32 days existed between cluster XXII (89 days) and cluster XV (121 days) in maturity duration. Similarly, plant height ranged from 60.99 (cluster XIII) to 100.82 cm (cluster XIX), and the number of productive tillers per plant varied from 2.40 (cluster II) to 3.65 (cluster XIV). Larger variation was also noticed for many traits, *viz.*, number of fingers per ear, length of the ear, finger length, finger width, number of leaves per main tiller, and flag leaf length.

The number of fingers per ear varied from 5.30 (cluster XXI) to 10.30 (cluster XXII), ear length ranged from 6.52 (cluster XVII) to 15.18 cm (cluster XX), finger length ranged from 4.42 (cluster XVII) to 13.63 cm (cluster XX), and finger width ranged from 0.60 (cluster XIII) to 1.08 cm (cluster XXI). The number of leaves per main tiller varied from 9.10 (cluster XVII) to 25.60 (cluster XXII), flag leaf length ranged from 34.75 (cluster III) to 53.40 cm (cluster XVII) while flag leaf width ranged from 0.95 (XXII) to 1.49 cm (cluster XVII). Length from top node to leaf sheath junction ranged from 7.14 (cluster IX) to 13.80 cm (cluster XX), peduncle length ranged from 5.84 (cluster XII) to 12.87 cm (cluster XVII). There was not much variation in disease incidence. However, grain and fodder yields varied more in between clusters. The average grain yield of cluster V (5250 kg/ha) was almost four times that of cluster V (1222 kg/ha). Similarly, the fodder yield of cluster IV (10499 kg/ha) was double that of cluster IV (5166 kg/ha). The mean performance of the clusters for 19 characters showed that the cluster II, consisting of a single genotype (IC0475882) had the highest grain yield (5250 kg/ha) and was characterized by low disease incidence for leaf blast grade (1.05), neck blast (12.03%), finger blast (17.42%), and banded blight (17.96%). Next, higher grain yields were recorded by clusters IX and XXI. The solitary cluster XIII (IC0283853) recorded the lowest plant height, while the solitary cluster XX (IC0474034) recorded longer ear length (15.18 cm) and finger length (13.63 cm). The solitary cluster XXII consisting of genotype IC0474832 recorded a higher fodder yield (10499 kg/ha) and was also characterized by a greater number of leaves per main tiller (25.60), a number of fingers per ear (10.30), and at the same time, it recorded earliness to maturity with short plant height. All these traits make this cluster very diverse from many other clusters. The cluster XVII with a single genotype (IC0478862) recorded the highest flag leaf length (53.40), flag leaf width (1.49), and

peduncle length (12.87). High inter-cluster distance between clusters XXII and XVII can be evidenced because cluster XXII was characterized by early maturity, lower plant height, more number of leaves/plant, and a short and narrow flag leaf along with high fodder yield. In contrast, cluster XVII was with medium maturity, fewer fingers per ear, fewer leaves per main tiller but with a longer and broader flag leaf. The genotypes from these clusters can be used as diverse sources in future breeding programmes. Crossing may bring together the advantageous features of a single genotype, resulting in a larger heterosis because genotypes from both clusters recorded comparatively better yields and each one has individual merit.

Because each cluster is characterized by specific characters based on the improvement goal, genotypes from different clusters can be chosen while keeping the inter-cluster distance in mind. For example, to have a high yielding variety with low disease incidence, genotype IC0475882 from cluster II can be chosen and can be directly used in evaluation trials. In order to get high-yielding, disease-resistant early maturing variety, one has to opt for crossing either IC0329452 from cluster IX, IC043734 from cluster XXI or IC0475882 from cluster II with IC0474832 from cluster XXII, having inter-cluster distances of 84.67, 74.27, and 68.22, respectively. To develop a high-yielding, non-lodging, early

Table 7: Relative contribution of 19 characters towards divergence in finger millet [*Eleusine coracana* (L.) Gaertn.]

S. No	Character	Times ranked	Contribution%
1	Grain yield	1172	10.92%
2	Days to 50% flowering	2036	18.97%
3	Days to maturity	79	0.74%
4	Plant height	667	6.22%
5	Number of productive tillers	211	1.97%
6	Number of fingers per ear	244	2.27%
7	Ear length	2211	20.60%
8	Finger length	71	0.66%
9	Finger width	487	4.54%
10	Number of leaves per main tiller	647	6.03%
11	Flag leaf length	504	4.70%
12	Flag leaf width	226	2.11%
13	Node to leaf sheath junction	218	2.03%
14	Peduncle length	1058	9.86%
15	Fodder yield	405	3.77%
16	Leaf blast	76	0.71%
17	Neck blast	208	1.94%
18	Finger blast	165	1.54%
19	Banded blight	46	0.43%

maturing variety, a cross between IC0474832 from cluster XXII and IC028353 from cluster XIII will be more effective with an inter-cluster distance of 91.36. Similar results were obtained by Kumar *et al.*, (2010), Rani *et al.*, (2014), Kumari and Singh (2015).

The percent contribution to genetic divergence was computed based on the number of times each of the 19 characters occurred in first place (Table 7). The results showed that the contribution of ear length per plant was the highest towards genetic divergence (20.60) by taking 2211 times to rank first. Similar results were reported by Mahanthesha *et al.*, 2017 and Devaliya *et al.*, 2017. Days to 50% flowering (18.97%) by 2036, grain yield (10.92%) by 1172 times. This implies that a lot of variation existed for ear length and days to 50% flowering while there was no variation for disease incidence.

Conclusion

It was observed that solitary cluster II consisted of a genotype, IC0475882, with high yield and disease resistance, which can be utilized directly after proper evaluation trials. To judiciously combine all the targeted traits into a single genotype, one has to go for hybridization between the selected genotypes from divergent clusters. On the basis of inter-cluster distances and mean values of clusters, crosses can be effected between IC0474832 and IC0478862; IC0329452 and IC0474832; IC043734 and IC0474832; IC0475882 and IC0474832; IC0474832 and IC028353 to have higher grain and fodder yield, disease resistance, earliness, and less plant height.

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RESEARCH ARTICLE

Efforts for the Revival of *Vigna subterranea* (L.) Verdc. (Bambara groundnut) through Germplasm Introduction in India

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Abstract

Vigna subterranea (L.) Verdc. (Bambara groundnut), an underutilized leguminous crop was studied with respect to plant morphology, prospects of crop introduction, and application of climatic analogues tools (CAT) to identify the most suitable areas for cultivation in India. Data on regular introductions of the crop in India was presented and the lack of information on crop performance in multi-location trials were the major gaps that need special attention in the Indian context. This paper attempts on some preliminary morphological observations under field trials and application of CAT besides botany, methods of propagation, use, and cultivation practices.

Keywords: Climate analogs tools, Crop introduction, *Vigna subterranea*.

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Introduction

Vigna subterranea (L.) Verdc. (family Leguminosae/Fabaceae) is among the most of the African crop species viz. groundnut (*Arachis hypogaea* L.), cowpea (*Vigna unguiculata* L.) that have contributed towards food, nutritional, and health security for humankind (Alhassan and Egbe, 2013). It is commonly known by several names- Bambara groundnut, Bambara bean, Bambara nut, hog-peanut, ground-bean earth pea, etc. Bambara groundnut is related to cowpea (*Vigna unguiculata*) and desires the same niche as the groundnut (*Arachis hypogaea* L.). The taxon seed is closer to chickpea (*Cicer arietinum*) (Halimi et al. 2019). The crop is extensively grown the sub-Saharan region of West Africa where it is considered as the third most important grain legume (Muhammad et al., 2020; Tan et al., 2020).

The sub-Saharan African warm tropical regions are the areas where bambara groundnut is mostly cultivated (Bamshaiye et al. 2011; Hillocks et al., 2012). Despite its drought-tolerant trait, it remains an underutilized legume only as a subsistence crop. In recent years bambara groundnut has attained the status of "wonder crop" due to its rich genetic diversity and potential in climate change regime (Muhammad et al., 2020).

The primary centre of diversity for bambara groundnut is in the north-eastern part of Nigeria and northern Cameroon. It is an underutilized legume assuming the status of nutritionally rich crops (Halimi et al., 2019) with high potential as biofertilizer in parts of Thailand and Indonesia. The crop is highly tolerant to biotic and abiotic stress condition such as drought, pests, diseases and well adapted to nutritionally poor soils and thus can secure production

under climate change regime. There is an extensive genetic variation in bambara groundnut for several agronomic characteristics and there is scope for improvement through breeding, value addition and marketing. The landraces of bambara groundnut are highly variable in seed testa colour and taste ranging from cream to white based on consumer preferences. Uba *et al.* (2021) have identified different landraces sub-population from different geographical regions. The seeds are a source of cheap and good quality protein and have been a food resource for poor people residing in semi-arid regions (Okonkwo and Opara, 2010; Tan *et al.* 2020). Since its introduction in many parts of the globe, the crop has gained significant consideration by the scientific and financial institutions at international levels but been largely ignored by the research community (Oyeyinka *et al.*, 2015) probably due to low prioritization crop at the institute level, poor policy decisions on crop implementation, acceptance by the farmers, non-availability of agronomic practices for crop cultivation (Hillocks *et al.*, 2012; Feldman *et al.*, 2019).

Subsequent upon introduction of bambara groundnut in India long back, research trials were conducted in several agro-climatic zones of the country. In recent efforts by the Indian Council of Agricultural Research (ICAR), National Bureau of Plant Genetic Resources (NBPGR), New Delhi ten accessions of bambara groundnut were introduced from Mali for adaptation trials under the collaborative programme on "Evaluation of Stress Tolerant Orphan Legumes (STOL) for Use in Dryland Farming Systems across Sub-Saharan Africa and India".

This paper attempts to report results of a preliminary experimental study on introduced germplasm of bambara groundnut from Mali to adaptation trials and use of climatic analogues to identify most suitable areas of crop introduction. Also information on potential uses of the crop, botany and propagation, cultivation etc. are provided with the perspective on recent research progress evaluating for future breeding in the Indian context.

Materials and Methods

Experiment and Planting Material

All 10 accessions of bambara groundnut imported from Mali were grown in Kharif 2020-21 at the NBPGR Pusa farm located in New Delhi. Preliminary morphological and evaluation data were recorded using selected accessions- EC1036859, EC1036855, EC1036861, EC1036862, EC1036853, EC1036854, EC1036860, EC1036857, EC1036858 and EC1036856. Data on various selected parameters were recorded for plants sown in a row of 2 m in length as per the available descriptors (IPGRI, IITA, BAMNET, 2000).

Data on previous introductions made in bambara groundnut in India were drawn from the records maintained in the Germplasm Exchange and Policy Unit located at

the ICAR-NBPGR, New Delhi. The climatic analogues data were gathered from areas of global cultivation and their equivalent climatic analogues zones in India and cast as sites of probable successful introduction in India. The climate gradient maps were casted based on data from literature survey, and climatic variables (*Worldclim.com*) and DIVA-GIS interfaced with climate data for precipitation and temperature gradients (Hijmans *et al.*, 2007; Semwal *et al.*, 2021). Information on botany, cultivation, propagation and uses were drawn through the literature available on the current status of crop in India and personal interaction with the indenters.

Result and Discussion

Morphological Observations

The bambara groundnut, an annual, is a short, creeping herb with a habit of bunchy and semi-bunchy form. The terminal leaflet is oval-shaped, measured from 5.1-6.4 x 1.8-2.1 cm, and the stem is densely pubescent. Usually, the petiole is longer than the leaflet and measured from 10-12.1 cm. The flower was mostly yellow (Fig. 1), measured from 0.8-1.3 cm. Vertical stripes are found in the standard petal (SP). After pollination, white coloured peg formation was initiated under the soil. Variation was recorded in mature pods from yellowish-reddish-brown (little grooves) ranging from 0.5-1.5 x 0.3-0.9 cm; seed variation was recorded for coat colour from cream (dark-red butterfly-like eye spots) to cream (black triangular eye-spot), 100 seed weight from 20-57 g and seed size from 0.5-1.1 cm.

Botany, Plant Propagation and Crop Production

The pods formation is alike peanut where pods develop underground; each pod has 1-2 seeds of round or unilaterally flattened shape with smooth hard seed coat, nearly 1.6 cm diameter, colour ranging from white, cream or brown, red, mottled and black. The plant needs a temperature in between 30-40°C at the time of flowering. The species is predominantly self-pollinated with cleistogamous flowers; flowering occurs in 40 to 60 days after planting. However, there are reports on generative autogamous (self-fertilization) and cleistogamous (self-pollinating) reproduction (Chandra *et al.*, 2020) of the species. The plant has 90 to 170 days growth cycle; pod maturity after 30 days of pollination.

Bambara groundnut in the Sahara region of Africa is an important food crop that grows successfully on diverse types of soil under hot, humid, cool climate (Temagne *et al.*, 2018). The crop is suitable for marginal soils and well adaptable to high temperatures.

The landraces of bambara groundnut are year old selections and culturally raised as solo crop or under intercropping with maize, millets, sorghum, peanut, yams, cassava, etc. having drought tolerance (Zeven, 1998; Olayide *et al.*, 2018). The yield (t ha⁻¹) is variable among the landraces

and areas of cultivation (Begemann, 1988). Over a period of more than three decades, the bambara groundnut world production has increased several folds and now amounting to 0.23 million tonnes annually in 2020 with a yield of 649.9 kg/ha (FAOSTAT, 2022).

Crop Introduction in India: Efforts in the Past

Bambara groundnut grows in all agro-climatic conditions where groundnut (*Arachis hypogea*; peanut) is cultivated (Singh *et al.*, 2020). In the Indian context a, limited research progress has been observed despite of its repeated introduction five decades back. Efforts were made to introduce *Vigna subterranea* in many Asian countries with identical agro-climatic conditions. This species was introduced to India and desirable germplasm was diffused to the indenters for research requirements. Analysis of the preliminary records on the exchange of the germplasm suggested that introductions trial for diverse seeded types in bambara groundnut were attempted in India as early as 1949 from parts of East Africa. Subsequent introductions were made in 1950, 1953, and 1957 from Australia, the

Department of Agriculture, Mauritius and others (Table 1). In 2019, under the exchange program by the ICAR-NBPGR fresh introductions from Mali were made for research under STOL project.

In literature, bambara groundnut is frequently reported under cultivation in tropical Central and South America, the Philippines, South Pacific, Australia and Papua New Guinea, southeast Asia India, Sri Lanka, Malaysia and Indonesia and grouped under the “secondary centre of diversity outside Africa” (Duke 1982; NAS 1979; Nwokolo 1996; Majola *et al.* 2021). Scrutiny of the Indian literatures as well as personal interaction with scientists working on crop confirmed that except the experimental cultivation, the crop had never been in public domain. However, reports on adaptive studies during the initial phase of the crop introduction in the country confirm experimental studies (Gowda and Krishnan 1968; Sanjappa 2010).

Studies on Adaptive Trials

A total of 12 varieties of bambara groundnut were tested in a replicated trial in Bhubaneswar, Odisha. Trial data revealed

Table 1: Import of bambara groundnut (*Vigna subterranea*) in India

EC No.	(year)	Source
EC1765-9	(1949)	East Africa (through Commission B.E.A)
EC1946	(1950)	Australia
EC4969	(1953)	Dept. of Agriculture, Ministry of Agro-Industry and Food Security Agricultural services, Reduit, Mauritius
EC12317-30	(1957)	Ministry of Agri., Shambat, Sudan
EC27879-81	(1964)	University College of Ghana , Achimota, ACCRA Ghana
EC37782-5	(1966)	ShesSector, Agricule, Cenbio, IRAI, BP-6, MARAD, NIGER
EC38245-50	(1966)	Dept. of Agriculture, Kampala, Uganda
EC38886-91	(1967)	The Director, IRAI, Senegal
EC42581-5	(1967)	Causeway, Salbury Rhodesia
EC10046-50	(1972)	Crops Research Institute, Bunso-Bosuso, Ghana
EC116163-164	(1976)	Rothamsted, England,
EC132769-770	(1980)	Mali
EC134501- 84		Nigeria
EC138348-53	(1980)	Agronomy Institute causeway, Zimbabwe
EC155891-2	(1983)	USDA-ARS Regional Plant Introduction Station, Georgia, USA
EC159150	(1984)	USDA-ARS Regional Plant Introduction Station, Georgia, USA
EC173345- 54	(1985)	Zimbabwe
EC191543- 7	(1986)	ICRISAT, Patancheru
EC240614-15	(1988)	UK
EC388662	(1996)	USDA-ARS, Beltsville, MD, USA
EC508312- 21	(2002)	Division of Agricultural Sciences, Leicestershire, UK
EC571836- EC571841	(2006)	Division of Agricultural Sciences, Leicestershire, UK
EC612805-806	(2008)	Ghana
EC698896- 8983	(2011)	IITA, Nigeria
EC1036853-58, 60-62	(2019)	Mali

that the range of variation was 53–59 for 50% flowering; 10.7–28 for plant stand at harvest, 34.4–38.2 cm for plant height; 11.6–26.6 for pods per plant and 0.62–2.69 q/ha for yield. Highest yield was in EC38245, followed by EC134535 (2.26 q/ha), EC37527 (2.13 q/ha) and EC134507 (2.01 q/ha) (Annual Report 1990-91).

Research attempts were made by ICAR-Directorate of Groundnut Research (formerly known as National Research Centre for Groundnut, NRCG), Junagarh, Gujarat, India to screen landraces of bambara groundnut for drought tolerance stress genes and for use in improvement in the groundnut. Singh and Basu (2006) while working on the physiology of bambara groundnut showed that the crop performance in Gujarat (western India) under a semi-arid climate and *kharif* season with early July sowing was most satisfactory. During the study three high-yielding genotypes DODR-TZ, SB 4-2 and S19-3 with 117-133 days maturity period and two short duration genotypes- AS-17, and S19-3 were identified. These were further tested in the Rajasthan, Maharashtra and Gujarat where the length of growing period was short.

Research studies undertaken in collaborative mode with NRCG, Junagarh, Gujarat; CAZRI, Jodhpur; RRS Bikaner, Rajasthan and University of Agricultural Sciences, Bangalore have suggested this crop can grow well in arid and semi-arid zones of Gujarat, Rajasthan and part of Andhra Pradesh (Bannayan *et al.*, 2000). Recent research initiatives of the ICAR under a collaborative programme entitled "Evaluation of Stress Tolerant Orphan Legumes (STOL) for use in dryland farming systems across sub-Saharan Africa and India- Promoting India-Africa Framework for Strategic Cooperation" resulted in the introduction of 10 accessions of very distinct germplasm and varietal material with a partnership with the Kirkhouse Trust (KT). The germplasm has been under detailed evaluation study at NBPGR, New Delhi, since 2020.

Use of Climatic Analogues Tools (CAT)

Reduced and erratic global rainfall has adversely affected the agricultural systems (IPCC 2014; Padulosi *et al.* 2011; Mayes *et al.* 2019) that has led to the identification of crops like bambara groundnut which can provide nutritional and food security. Production of bambara groundnut needs optimal temperature between 19–30°C, minimal annual rainfall about 300 mm and optimal annual rainfall between 750–1400 mm.

Success of introduced germplasm is assessed by the effective response of species performance under new environments and areas of better adaptation. In the present study, the crop analogue sites (called reference sites) were identified with help of Climate Analogue Tool (CAT) tools. Identification of suitable analogue sites for developed varieties and newly introduced germplasm based on the characteristics of the site is possible through CAT application (Mubaiwa *et al.*, 2018).

Bannayan *et al.* (2000) undertook field studies and suggested areas in Gujarat (Western India) and Andhra Pradesh (Southern India) for multi-locational testing and release of promising accessions in India. Study resulted in identification of suitable sites in different states (districts in parenthesis) in the country through climatic gradient data from the sites of cultivation superimposed on the similar sites in the Indian region were drawn and - Gujarat Krishnagiri, Dharmapuri, Salem, Villupuram) (Figure 1 and 2). Field studies and adaptive trials on the bambara groundnut identified potential in semi-arid tropics of India with moderate suitability for cultivation of bambara groundnut (Singh and Basu, 2006).

Bambara groundnut popularization and commercialization will be based on the nutritional aspects and its performance in different areas. Government intervention and networking through AICRN on potential crops has to play a pivotal role in multi-locational trials in most suitable areas and has to come up with packages of practice for this crop.

Uses

In India, bambara groundnut is still in the trial stage. The crop is extensively used in African regions in roasted form, as a snack, processed into cake, in boiled form.

The flour is suitable for preparing cakes, puddings, bread, etc. A delicacy, "Okpa", known from South Eastern

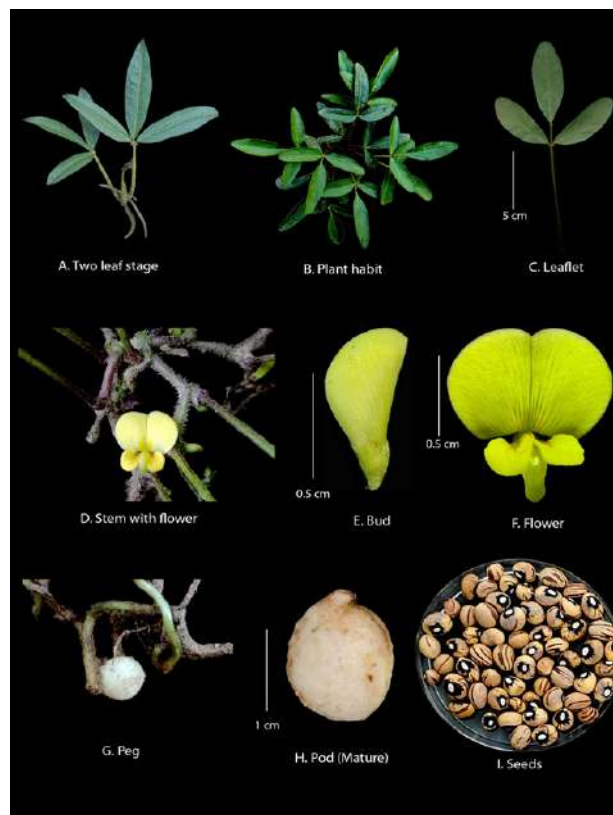


Figure 1: Depiction of vegetative and reproductive parts of bambara groundnut.

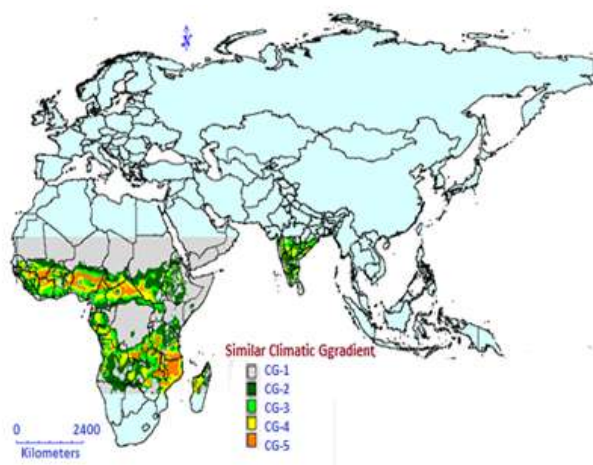


Figure 2: Climatic analogous sites (African continent) identified with current climate of Indian states, indicating potential options for exchange of bambara: CG-1-Climature gradient -1 (unsuitable); CG-2-Climature gradient -2 (low suitable); CG-3-Climature gradient -3 (medium suitable); CG-4-Climature gradient -4 (high suitable); CG-5-Climature gradient -5 (very high suitable).

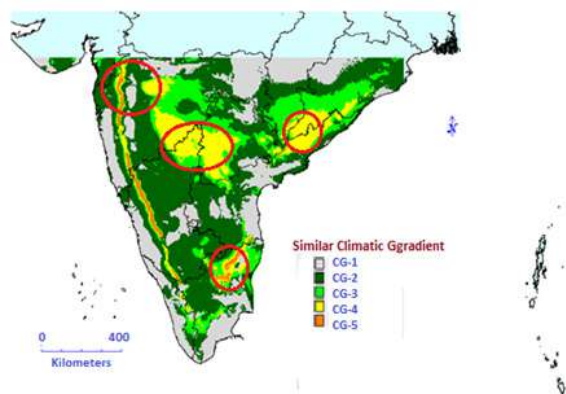


Figure 3: Climatic analogous sites with the current climate of Indian states, indicating potential options for exchange of bambara groundnut: CG-1-Climature gradient-1 (unsuitable); CG-2-Climature gradient-2 (low suitable); CG-3-Climature gradient -3 (medium suitable); CG-4-Climature gradient -4 (high suitable); CG-5-Climature gradient -5 (very high suitable shown with circles).

Nigeria, is prepared using finely powdered beans of bambara groundnut mixed with palm oil, water and pumpkin leaves turned into a paste and then boiled after wrapping into banana leaf.

Conclusions

The bambara groundnut could provide quality plant proteins and has great potential for use in various forms as human diet, especially in climate change-ready agriculture where other protein-rich crops may not perform well. However, the crop is under-researched and under-utilized in many parts of the globe; data on yield, proper package of practices, application of nutrients and improvement of the nitrogen-fixing capacity and studies on crop phenology are meager. India looks forward with the possibility of

bambara groundnut as an important resource for plant genetic resources.

New generations of bambara groundnut cultivars should comprise of market preference, including desirable agronomic, nutritional, and processing qualities that benefit the food and feed industry. The study brings out the following bottleneck points that emerge in the Indian context:

- Data on performance and pre-evaluation trials in public domain to facilitate gaps and thrust in genetic improvement of the crop.
- Information on the genetic diversity, drought tolerance, and nutrient contents of diverse collections.
- Conducting multi-location trials through All India Coordinated Research Project and performance and most suitable growing period.
- Availability of diverse germplasm in genebanks for future research and developments.
- Adaptive trials after using CAT tools.

Like other under-utilised crops, unsuitability of Bambara groundnut as food due to hardness of seed and 'photoperiod sensitivity to pod filling' are associated with the crop use and deserve to be thrusts in breeding programmes. This crop deserves its prime role in broadening India's food basket base. The authors are engaged in further studies under evaluation programme at the NBPGR, New Delhi.

Acknowledgements

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Plant Germplasm Registration Notice*

The Plant Germplasm Registration Committee of ICAR in its XXXXIIIrd meeting held on March 18th, 2021 at the National Bureau of Plant Genetic Resources, New Delhi. A total of 125 proposals were received for registration. Out of that, 105 proposals were considered for registration. Finally, 90 applications covering 35 crop species were approved for registration. The information on registered germplasm is published with the purpose to disseminate the information to respective breeders for utilization of these genetic stocks in their crop improvement programmes. Upon request, the developer (s) / author (s) is / are obliged to distribute the material for crop improvement programme of National Agricultural Research System.

1. Pant CMS3A & Pant CMS3B (IC0635012 & IC0635013; INGR21001), a Rice (*Oryza sativa*) Germplasm with Fully Exserted Panicle. High Number (305.31) of Grains Per Panicle. Excellent Outcrossing Rate

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Hybrid rice cultivation is gaining world wide popularity. Research on hybrid rice in China was initiated in 1964 (Yuan, 1966). The genetic tools essential for breeding hybrid rice varieties are as the male sterile line (A-line), maintainer lines (B-line) and restorer (R-line) were developed during 1973 (Yuan and Virmani, 1988). Use of gibberellic acid (GA3) at proper stage with proper dose enhances the seed set on seed parent (Virmani, 2005), but the use of GA3 also enhances the cost of seed that farmers or seed producers has to bear. The cost of one gram of GA3 in the local market varies between Rs. 120-150 depending upon the purity and company. With a view to improve the seed set of CMS lines in rice, a conversion programme in the background of an improved rice genotype, "NAT 990-99" was initiated at GB Pant University in 2003 by using widely used CMS line "IR58025A" as non recurrent parent. Progenies in each backcross generation were checked for pollen sterility and morphological traits. In BC6 generation progeny with desired morphological traits, that is fully exserted panicles, complete sterile plants were selected and allowed to cross pollination with the maintainer line NAT 990-99 in isolation, to get the genetically pure seed of cytoplasmic male sterile line. The conversion procedure was followed as in the development of Pant CMS 2A (Nautiyal *et al.*, 2011).

Morpho-agronomic characteristic: Newly developed PantCMS3A has got complete exserted panicle with the

dark green leaves plant height 120-125 cm with sturdy stem which prevents the lodging the crop. Yield (kg/ha) was found maximum with mean value in Pant CMS3A 2043.00 kg/ha in natural conditions without using supplementary pollination in four years.

Associated characters and cultivation practices: Newly developed Cytoplasmic Genetic Male Sterile Line Pant CMS3A has 12-15 effective tillers per plant. The leaves are dark green. It is moderately resistant to bacterial leaf blight disease and tolerant to stem borer. It can be cultivated in the rice growing areas under irrigated under high fertility conditions.

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Table 1: Panicle length, Number of filled grains, Total number of grains and Yield (Kg/ha) in different CMS lines

CMS Line	Panicle length (cm)				Number of filled grains				Total number of grains				Yield (kg/ha)							
	2011	2012	2013	2014	Mean	2011	2012	2013	2014	Mean	2011	2012	2013	2014	Mean	2011	2012	2013	2014	Mean
IR58 025 A	20.66	21.66	20.88	21.66	21.21	23.36	27.30	23.86	27.60	25.53	3	6	3	6	2	0	0	1178.00	1231.0	1204.0
IR58 025 B	21.46	21.90	21.34	21.64	21.58	147.1	154.2	148.0	154.0	150.8	168.1	171.4	170.1	172.4	170.6	2616.0	2693.0	2700.0	2764.0	2693.2
Pusa 6A	24.7	24.9	24.80	24.9	25.5	24.72	28.90	24.20	29.00	26.70	136.4	142.0	138.6	140.0	139.2	1153.0	1193.0	1155.0	1196.0	1174.2
Pusa 6B	25	26	25.83	26	26	13	15	13	16	14	15	16	15	16	16	239	248	249	242	244
	.6	.5		.5	.1	7.8	5.1	8.0	0.0	7.7	0.4	8.6	4.6	9.0	0.6	3.0	3.0	2.0	0.0	7.0
	3	6		4	4	3	6	0	0	4	0	6	0	0	6	0	0	0	0	0
Pant CMS 3A	29	30	29.30	30	29	10	11	11	11	11	34	34	33	34	34	195	204	205	204	202
	.1	.6		.6	.9	9.2	0.5	1.0	3.2	0.9	0.2	9.8	8.4	8.2	4.1	3.0	3.0	3.0	3.0	3.0
	3	6		6	3	0	0	0	5	8	6	3	0	0	7	0	0	0	0	0
Pant CMS 3B	31	32	31.83	32	32	30	30	30	31	30	32	32	32	32	32	463	483	483	502	483
	.4	.6		.4	.0	5.4	3.8	2.0	0.0	5.3	1.4	2.3	4.6	6.2	3.6	3.0	3.0	3.0	2.0	0.2
	3	0		0	6	3	3	0	0	1	0	3	0	0	3	0	0	0	0	5
IR79 156 A	22	23	23.34	23	22	24	29	29	28	27	17	18	17	17	17	116	118	119	126	120
	.4	.0		.0	.9	96	00	20	60	94	8.2	0.2	6.3	9.2	8.4	8.0	0.0	9.0	5.0	3.0
	6	0		0	5						3	0	3	0	9	0	0	0	0	0
IR79 156 B	21	23	23.84	24	23	15	15	16	15	15	17	18	17	18	17	289	276	279	269	278
	.8	.2		.2	.2	8.6	9.8	0.0	8.0	9.1	9.2	0.2	8.4	0.2	9.5	9.0	2.0	2.0	2.0	6.2
	0	6		0	7	0	0	0	0		3	1	0	0	1	0	0	0	0	5
CD at 5%	1	0	1.20	0	-	7.1	4.0	7.1	4.0	-	4.7	1.9	4.7	1.9	-	1.9	1.6	1.9	1.6	-
	22	95		92		3	7	1	6		7	6	8	2		2	5	1	1	
CV	2	1	2.60	1	-	3.1	1.7	3.1	1.7	-	1.2	0.4	1.2	0.4	-	4.5	3.7	4.4	3.7	-
	63	98		96		4	2	2	0		2	8	0	9		4	9	9	2	

2. AC 42997 (IC0576152; INGR21002), a Rice (*Oryza sativa*) Germplasm with Vegetative Stage Drought Tolerance. Prolific Roots. High Water Use Efficiency.

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The germplasm line (AC 42997) was collected and conserved at NRRI Rice gene bank. This showed tolerance to vegetative stage drought stress consistently for five years (2011 onwards) after rigorous screening under simulated moisture stress in NRRI. Under simulated stress during vegetative stage, AC 42997 was observed to be tolerant with least leaf rolling SES "1", leaf death score of SES "3", had high root length density, root dry weight and root to shoot dry weight ratio (CRRRI Ann. Rep 2012-13, pp- 97; Dash *et al.* 2017), high water retention capacity (>70%), at 10-13% soil moisture content and soil moisture tension of -55 to -60 kPa during the stress period. The germplasm line was tested in NRRI, Cuttack from 2011-2016 for multiple years under NICRA Project. It is

identified promising and unique with many traits compared to other genotypes.

It has high *per se* performance for more than one root traits under control and stress condition. Though many germplasm lines have been identified for drought tolerance, there is also urgency to identify more and more donors with specific traits to evolve better varieties with higher drought tolerance coupled with higher grain yields, in view of the fast-changing environments. Same level of resistance does not mean the gene (s) responsible for tolerance will be same. Hence this germplasm line/landrace may be registered as a donor for vegetative stage drought tolerance with specific root traits responsible for drought tolerance.

Table 1: Performance of AC42997 for different drought related physiological traits

Traits	Significance	Reference
Drought score	Resistant (R), SES "3"	Dash <i>et al.</i> , 2017
Root Traits	Root and shoot length: Increase in maximum root length under drought Root thickness: Moderate Root length density: high Root biomass: High Root to shoot dry wt ratio: High under drought compared to control root volume: high Root length density: high	Dash <i>et al.</i> , 2017, Acta Physiol. Plant (2017) 39:8 DOI10.1007/s11738-016-2297-1 CRRRI Annual Report 2012-13, Pg No. 97 ARRW Golden Jubilee International Symposium on "Sustainable rice production and livelihood security: challenges and opportunities" held during 2-5 March 2013 at Central Rice Research Institute, Cuttack, Odisha. Pg No. 262-263. National Conference of Plant Physiology (NCP) on "Frontiers of plant physiology research: food security and environmental challenges" held during 23-25 Nov 2014 at OUAT, Bhubaneswar, Odisha. Pg No. 226.
Germination percentage	High percent of germination (>90%) under 1% and 2% mannitol induced osmotic stress.	Dash GK and Swain P (2015). <i>Oryza</i> 52 (4): 307-312.
Water use efficiency	High water use efficiency (3.26 g biomass/kg water) under drought stress and well-watered (2.55 g biomass/kg water) condition.	International Rice Symposium on "Rice Science for Global Food and Nutritional Security" held during 18-20 th Nov 2015 at Hyderabad. Pg No. 238
Transpiration rate	Slow transpiration rate per unit leaf area under well-watered (3.69 g/cm ²) and water stressed condition (3.11 g/cm ²)	International Rice Symposium 2015 on "Rice Science for Global Food and Nutritional Security" held on 18-20 th Nov 2015 at Hyderabad. Pg No. 238
Stomatal density	Low stomatal density (352.2/mm ²)	International Rice Symposium (IRS) 2015 on "Rice Science for Global food and Nutritional Security" held during 18-20 th Nov 2015 at Hyderabad. Pg No. 238

3. IC330611 (IC0330611; INGR21003), a Rice (*Oryza nivara*) Germplasm Vegetative stage drought tolerance.

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The wild species of the cultivated rice are a good reservoir of genetic variability for various biotic and abiotic characters. They harbour significantly higher genetic diversity than the cultivated species. During 1999-2001, a total of 483 accessions of two common wild rice species, namely *Oryza nivara* and *O. rufipogon* were collected from south east India – where they occur abundantly. On evaluation against abiotic stress specifically to drought, two accessions of *O. nivara* (IC330611) were found tolerant to moisture stress with SES score of 0 & 1. These germplasms were collected from West Bengal and were screened under field condition at NRRI during dry season of 2003 & 2004 in augmented design. Five checks viz. Salumpikit, Vandana, Vanaprabha and CR 143-2-2 as tolerant and IR 20 as susceptible check were used during screening trial. Plants were grown for 35 days after germination with sprinkler irrigation. The irrigation was withdrawn for 30 or more days till the susceptible check showed permanent wilting followed by watering for recovery. Peizometers were fixed to monitor ground water table at 15, 30 and 45 cm depth at periodic intervals after suspension of sprinkler irrigation. Ground water table was below 100 cm during the period of drought stress and soil moisture decreased from 30 to 5% up to 45 cm soil depth. The drought scores and recovery data was taken as per SES method (1 to 9 scale). The lines with early leaf rolling after

suspension of sprinkler irrigation showed higher score for drought tolerance (7-9) and lines with delayed leaf rolling recovered faster after rewatering. The scoring of this tolerant wild rice is 1 in SES scale.

It is of significance in the context of drought situation, as the habitat of wild rice species is usually swampy places like seasonal ditches. Root length of *O. nivara* was 20.2 cm under normal condition while it was 25.7 cm under moisture stress. Root penetration in *O. nivara*, an annual wild rice species was deeper while in *O. rufipogon*, a perennial wild species, it was shallow and adventitious. Under simulated stress during vegetative stage, it is observed to be tolerant with least leaf rolling SES "1", leaf death score of SES "3" had high root length density, root dry weight and root to shoot dry weight ratio, high water retention capacity (>70%), at 10-13% soil moisture content and soil moisture tension of -55 to -60 kPa. Though many germplasm lines have been identified for drought tolerance, there is still urgency to identify more and more donors with specific traits to evolve better varieties with higher drought tolerance in view of the fast-changing environments. Same level of resistance does not mean the gene (s) responsible for tolerance will be same. Hence this germplasm line/landrace can be used as a donor for vegetative stage drought tolerance.

4. IC330470 (IC0330470; INGR21004), a Rice (*Oryza nivara*) Germplasm with Vegetative Stage Drought Tolerance

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at NRRI during dry season of 2003 & 2004 in augmented design. Five checks viz. Salumpikit, Vandana, Vanaprabha and CR 143-2-2 as tolerant and IR 20 as susceptible check were used during screening trial. Plants were grown for 35 days after germination with sprinkler irrigation. The irrigation was withdrawn for 30 or more days till the susceptible check showed permanent wilting followed by watering for recovery. Peizometers were fixed to monitor ground water table at 15, 30 and 45 cm depth at periodic intervals after suspension of sprinkler irrigation. Ground water table was below 100 cm during the period of drought

stress and soil moisture decreased from 30 to 5% up to 45 cm soil depth. The drought scores and recovery data was taken as per SES method (1 to 9 scale). The lines with early leafrolling after suspension of sprinkler irrigation showed higher score for drought tolerance (7- 9) and lines with delayed leaf rolling recovered faster after rewatering. The scoring of this tolerant wild rice is 1 in SES scale.

It is of significance in the context of drought situation, as the habitat of wild rice species is usually swampy places like seasonal ditches. Root penetration in *O. nivara*, an annual wild rice species was deeper while in *O. rufipogon*, a perennial wild species, it was shallow and adventitious.

Under simulated stress during vegetative stage, it is observed to be tolerant with least leaf rolling SES "1", leaf death score of SES "3" with high water retention capacity (>70%), at 10-13% soil moisture content and soil moisture tension of -55 to -60 kPa. Though many germplasm lines have been identified for drought tolerance, there is still urgency to identify more and more donors with specific traits to evolve better varieties with higher drought tolerance in view of the fast-changing environments. Same level of resistance does not mean the gene (s) responsible for tolerance will be same. Hence this germplasm line/landrace can be used as a donor for vegetative stage drought tolerance.

5. Dubaraj (IC301206) (IC0301206; INGR21005), a Rice (*Oryza sativa*) Germplasm with Very High 1000-Grain Weight (50.4g).

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Rice is one of the major crops of India, where there is considerable range of variability exists. Its cultivation dates back to 6000 years ago, as it has its mention in ancient scriptures. The records claim more than 1,50,000 rice landraces which were in cultivation in India. Owing to the popularization of the fertilizer responsive, high yielding varieties, many of these landraces have vanished from the geography of India. However, the socio-economic, ethnic cultures prevailing around the rice cultivation and the thoughtful collection and conservation of the landrace diversity, more than 55,000 rice landraces are conserved in the National Genebank.

Apart from their remarkable adaptability to the local climate change, some of the landraces are known for their unique traits. Dubaraj is one such landrace collected from the Chattisgarh region, which is one of the aromatic rice. The characterization of the germplasm collection has shown that the one of the accessions of Dubaraj, IC 301206 which has its source from Raipur, Chattisgarh, has recorded high 1000 grain weight, i.e., 50.4g.

IC301206 is a long grain rice, whose morphological characters are given below

Characteristics	Description
Early Plant Vigour	Very Good
Coleoptile Colour	Green
Basal Leaf Sheath Colour	Green
Leaf Blade Colour	Green
Leaf Pubescence	Pubescent

Table continued...

Stigma Colour	White
Ligule Shape	2-Cleft
Auricle (Pres/Abs)	Green
Awning	Long fully awned
Panicle Type	Intermediate
Panicle Exsertion	Medium well exserted
Flag Leaf Angle	Erect
Apiculus Colour	White
Leaf Senescence	Slow
Panicle Threshability	Intermediate
Husk Colour	White
Sterile Lemma Colour	Straw
Kernel Colour	White
Aroma	No scent
Seedling Height (cm)	22.8
Leaf Length (cm)	58.0
Leaf Width	1.14
Days to 50% Flowering	131 days
No. of Effective Tillers	4.4
Plant Height (cm)	177.4
Panicle Length (cm)	29
Days to Maturity	162 days
Grain Length/Breadth	4.7
1000 grain weight (gm)	50.4
Grain yield per Plant (gm)	11.01

6. Karuppunel (IC0637545; INGR21006), a Rice (*Oryza sativa* var. *indica*) Germplasm with High Grain Zinc Content (41.05 ppm)

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It is accepted across the global scientific community that modern day rice cultivars are high yielding but low in micronutrient status which is predisposing rice eating population to micronutrient malnutrition. The situation is grim in poor and developing nations who cannot afford to diverse diets to meet their nutrient requirements. Hence rice is the choice crop for bio fortification programmes to address the hidden hunger among poor rural populations. The rich genetic diversity of rice preserved in landraces offer huge potential for improving the nutritional status of high yielding modern rice cultivars. Karuppunel is one such landrace which has huge potential for enriching the endosperm zinc content of popular rice varieties. The accession was collected from Tamil Nadu state, purified and being maintained as part of breeders collection in the Division of Genetics, ICAR-Indian Agricultural Research Institute (IARI), New Delhi.

Morpho-agronomic characteristics: Upon evaluation of a set of 192 rice accessions for grain iron and zinc concentrations, Karuppunel recorded as high as 46.2 ppm in brown rice and 40.9 ppm in polished rice (Bollinedi *et al.* 2020a & 2020b), the values significantly higher than the recommended target of 28 ppm for biofortification programmes as per the Harvest Plus guidelines (Bouis *et al.* 2011). The accession was further nominated for testing

at multiple locations under a biofortification donor trial conducted under the consortium research project (CRP) on Biofortification and it out-performed all the promising donors in the trial with a range of 29.1 ppm to 49.67 across the environments. It has also recorded considerably high mean concentration of 4.3 ppm iron in polished rice as against to 2 ppm in popular cultivars. The other agro- morphological characteristics of Karuppunel are plant height (146 cm), days to 50% flowering (98 days), panicle length (23.2 cm). It has black husk and red pericarp due to accumulation proanthocyanidins with beneficial health impact.

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7. IET17948 (IC0637546; INGR21007), a Rice (*Oryza* sp.) Germplasm Bacterial Blight Resistant with Three Bacterial Blight Resistance Genes, *xa5*, *xa13* and *Xa21* Pyramided in PR106

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Bacterial blight (BB) of rice, caused by gram-negative bacteria *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the most destructive disease worldwide leading up to 60% of the yield loss under severe condition (Jiang *et al.* 2020). Pyramiding of two or more genes will be more effective in pathogen evolution at lower rate (Singh *et al.* 2001). Here, we want to register the rice line IET- 17948 (PR106 / IRBB62 // 2*PR106) with three bacterial blight resistance genes viz *xa5*, *xa13* and *Xa21* pyramided into an Indica rice cultivar PR106 at PAU. The crosses were made between high yielding bacterial blight susceptible cultivar PR106 and IRBB62 (having 3 resistance genes). F₁ plants were backcrossed with PR106

to generate BC₁F₁'s. PCR based molecular markers linked to *xa5*, *xa13* and *Xa21* resistance alleles were used to select plants from BC₁F₁ onwards. The BC₂F₃ lines having 2 or 3 homozygous genes for resistance to BB were identified on the basis of molecular marker analysis and inoculated with BB isolates/races to determine disease reaction. Genes in combinations were found to provide high levels of resistance to predominant Xoo isolates.

Morpho-agronomic characteristics: BC₂F₄ progenies were evaluated in commercial fields (Ludhiana, Sangrur, Jalandhar and Ferozpur) against the pathogen

populations prevalent in the region. Lines of PR106 with three pyramided genes were evaluated against 17 isolates from the Punjab and six races of *Xoo* from the Philippines under natural conditions at 31 sites. The lines with three genes showed superior level of resistance as compared to the lines with either single or double bacterial blight resistance genes.

Associated characters and cultivation practices: IET17948 carries three bacterial blight resistance genes which gives complete resistance at the seedling and adult plant stage to *Xanthomonas* pathotype seven.

8. ILPAUGS_BPH34 (IC0637548; INGR21008), a Rice (*Oryza* sp.) Germplasm Resistance against BPH Biotype 4 Prevalent in India carrying the novel Brown plant hopper resistant gene BPH 34 from *Oryza nivara* acc. IRGC104646 on rice chromosome 4

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Brown Planthopper (BPH, *Nilaparvata lugens* Stål) is one of the most destructive insects of rice (*Oryza sativa* L.) causing significant yield losses annually. Exploiting host plant resistance to BPH and incorporating resistant genes in susceptible commercial cultivars is economical and environmentally friendly approach to manage this pest. Here, we want to register a stable F8 recombinant inbred line, PAUGS_ BPH (34) with brown plant hopper resistance. This gene was introgressed from *Oryza nivara* accession IRGC104646 to the indica rice cultivar Punjab Rice 122 (Kishor *et al.*, 2017, Sarao *et al.*, 2016). This accession was found resistant against BPH biotype 4, prevalent in Indian subcontinent. Inheritance study using F₂ and F_{2:3} populations revealed the presence of single dominant gene. This novel BPH resistant gene was mapped on long arm of rice chromosome 4 and designated as *Bph34*. Two of the SSR markers RM16994 and RM17007 were found co-segregating with the *Bph34*. We have generated homozygous resistant F₉ progenies (PR122/ *O. nivara* IRGC 104646) with good agronomic background. This genetic stock could be used by plant breeders for further transfer of BPH resistance to other elite cultivars using marker assisted selection.

9. PAU_CB28 (PR114_Xa38) (IC0637549; INGR21009), a Rice (*Oryza sativa*) Germplasm Carries Bacterial Blight Resistance Gene from *Oryza nivara*

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Bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*) is one of the major constrain to the productivity in South-East Asia (Cheema *et al.*, 2008, Bhasin

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Morpho-agronomic characteristics: The Agro-morphological features include green sheath, acute-white ligule, erect leaf blade, white stigma, straight panicle, white lemma, yellowish white awns at tip and erect to semi-erect panicle branches on straight main axis of panicle.

Associated characters and cultivation practices: The genetic stock IL PAUGS_ BPH (34) has brown plant hopper resistance against BPH biotype 4 prevalent in India. Standard rice agronomic practices can be used.

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et al., 2012). An accession of *Oryza nivara* (IRGC 81825) was found resistant to all the seven *Xoo* pathotypes prevalent in northern states of India. The inheritance studies was

conducted using F₂, BC₂F₂, BC₃F₁ and BC₃F₂ progenies of the cross involving *Oryza sativa* cv PR114 and the *O. nivara* acc.81825 using the most virulent *Xoo* pathotype seven. Genetic analysis of the segregating progenies revealed that the BB resistance in *O. nivara* was conditioned by a single dominant gene. This novel bacterial blight resistance gene was mapped on long arm of chromosome 4 and designated as *Xa-38*. The PCR-based sequence-tagged site marker was developed for effective transfer of the *Xa-38* bacterial blight resistance gene. The homozygous resistant BC₃F₃ progenies with smallest introgression region have been identified and single seed decent method was used for the generation advancement till BC₃F₁₀. Here, we want to register PAU_CB28 (PR114/*O. nivara* IRGC81825//2*PR114) with bacterial blight resistance.

Morpho-agronomic characteristics: The Agro-morphological features include green basal leaf sheath color. Leaf pubescence of blade surface was absent. The shape and color of the ligule were acute and white, respectively. Early and late observation showed that the flag leaf (attitude of the blade) was erect. Spikelet density of pubescence of lemma was weak. Anthocyanin color of both lemma at the

apex and stem at the nodes was absent. Spikelet's color of stigma was white. The panicle curvature of the main axis was straight. The color of the tip of the lemma in the spikelet was white. The awns were absent or very small. The attitude of branches of panicle was erect to semi-erect.

Associated characters and cultivation practices: PAU_CB28 (PR114_ *Xa* 38) carries bacterial blight resistance gene *Xa38* from *Oryza nivara* acc. IRGC 81825 which gives complete resistance at the seedling and adult plant stage to *Xanthomonas* pathotype seven. Standard agronomic practices for rice growing can be used.

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10. IET19339 (IC0637550; INGR21010), a Rice (*Oryza* sp.) Germplasm IET19339 carries three Bacterial Blight Resistance Genes *xa5*, *xa13* and *Xa21*, Pyramided into cv. Pusa 44

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Bacterial blight (BB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is major yield limiting biotic stress in rice production in the Punjab state as rice is grown under irrigated and high fertilizer input conditions that are conducive to the disease development (Lore *et al.* 2011). The identification and exploitation of resistant germplasm is one of the effective and environmentally acceptable approaches to counteract the damage caused by this pathogen. Pyramiding of two or more genes will be more effective in pathogen evolution at lower rate. Here, we report the pyramiding of three bacterial blight resistance genes viz *xa5*, *xa13* and *Xa21* into *indica* rice cultivar Pusa 44 at PAU. F₁ plants were backcrossed with Pusa 44 to generate BC₁F₁'s. PCR based molecular markers linked to *xa5*, *xa13* and *Xa21* resistance alleles were used to select plants from BC₁F₁ onwards. The BC₂F₃ lines having 2 or 3 homozygous genes for resistance to BB were identified on the basis of molecular marker analysis and inoculated with BB isolates/races to determine disease reaction. We want to register a stable backcrossed introgression line with bacterial blight resistance. Genes in combinations were found to provide high levels of resistance to predominant *Xoo* isolates.

Morpho-agronomic characteristics: The basal leaf sheath color of plants was green. Leaf pubescence of blade surface

was absent. The shape and color of the ligule were acute and white respectively. Early and late observation showed that the flag leaf (attitude of the blade) was erect. Spikelet density of pubescence of lemma was weak. Anthocyanin color of both lemma at the apex and stem at the nodes was absent. Spikelet's color of stigma was white. The panicle curvature of the main axis was straight. The color of the tip of the lemma in the spikelet was white. Panicle awns were present. Late observation showed the yellowish-white color of awns and was present only at the tip. The attitude of branches of panicle was erect to semi-erect.

Associated characters and cultivation practices: The rice genetic stock IET19339 carries three bacterial blight pyramided resistance gene and provides complete resistance at the seedling and adult plant stage to *Xanthomonas* pathotype seven. Standard agronomic practices for rice growing can be used.

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11. EC670488 (EC670488; INGR21011), a Rice (*Oryza sativa* x *O. glaberrima*) Germplasm Tolerant to High Temperature Stress (>35°C) at Reproductive Stage with Very High Spikelet Fertility Particularly Under High Temperature Stress

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Climate change and global warming are posing a serious threat to agricultural productivity worldwide. India is projected to suffer from temperature spike to a tune of 4.7-5.5°C by 2100 paralleling global climate change. Rice is sensitive to heat stress especially at anthesis stage which will be directly reflected in significant grain yield reduction. A new reproductive stage heat stress tolerant (RSHT) genotype NERICA

L 44 (NL 44) shows tolerance levels almost at par with the upland rice variety, Nagina 22, which is one of the most reported for heat stress tolerance in rice. The unique nature of this genotype is, its peak anthesis falls around 10.00 am when the ambient temperature has already begun to raise making it a truly RSHT genotype. NL 44 is a NERICA rice variety developed from the inter-specific cross between *Oryza glaberrima* (African rice) and *O. sativa* (Asian rice). In our screening for reproductive stage heat stress tolerance, the rice genotypes, NL 44 consistently showed superior spikelet fertility and grain yield under heat stress evaluated at two locations, Aduthurai and Cuttack during two consecutive seasons (off-season) 2019-20 and 2020-21. The performance of NL 44 in comparison with popular checks in various heat stress trials are summarized in Table 1. The genotype maintained a spikelet fertility percentage of ~85% under heat stress (> 35°C) and grain yield plant⁻¹ of ~20g (Ravikiran *et al.* 2020). A representative picture showing the panicles and grains (filled and unfilled) under normal

(33–35°C) and heat stress (>35°C) in the heat stress tolerant rice genotype, NL44 and the sensitive rice variety, Pusa Basmati 1 is presented in Figure 1. Trait specific validation was carried out using 113 SSR markers linked to the 54 different QTLs previously reported for reproductive stage heat stress (RSHS) tolerance, identified NL44 as a novel donor for RSHS tolerance in rice (Ravikiran *et al.* 2020). Additionally, unlike N22, NL 44 possesses other desirable characteristics such as photo insensitivity, short stature, good grain quality attributes, etc. The grain quality characteristics of this genotype are acceptable (long slender with moderate amylose content) making it an invaluable donor to breed RSHT rice varieties.

The following are salient characteristics of this genotype:

- Under normal conditions shows a spikelet fertility ~ 90%, while under heat stress (>35°C) maintains 80-85% spikelet fertility
- Photo-insensitive, short stature (90-95 cm) and medium duration (days to 50% flowering – 105-110 days) genotype
- Well exerted, erect to semi-erect, long (28-30 cm) panicles (13-15 in number per plant) awnless grains
- Grain yield at normal temperatures is 26-28g per plant while under heat stress it is 19-22g.
- Long slender grain type with medium test weight (20-23g), elongation ratio (~1.57) and amylose content.

Table 1: Relative performance of NL 44 during off-season, 2018-19 and 2019-20 at two locations, Aduthurai and Cuttack

Season	Trait	Treatment	NL44	Nagina 22	IR64	Pusa Basmati 1
Off Season 2018-2019, Aduthurai	GYPP (g)	Control	25.75	18	25.5	26.05
		Heat Stress	20.64	17.45	14.4	14.79
	Spikelet Fertility (%)	Control	87.71	92.52	85.83	74.66
		Heat Stress	84.35	91.46	70.76	42.85
Off Season 2019-2020, Aduthurai	GYPP (g)	Control	24.33	17.05	26.84	22.85
		Heat Stress	19.3	12.58	12.3	8.76
	Spikelet Fertility (%)	Control	91.16	94.39	85.38	81.34
		Heat Stress	84.35	91.79	72.41	41.62
Off Season 2018-2019, Cuttack	GYPP (g)	Control	20.31	16.53	20	20.7
		Heat Stress	12.25	8.03	8.63	4.67
	Spikelet Fertility (%)	Control	84.08	90.58	84.08	85.88
		Heat Stress	78.23	89.15	62.37	45.61
Off Season 2019-2020, Cuttack	GYPP (g)	Control	25.66	18.27	25.27	23.86
		Heat Stress	85.7	92.41	91.12	71.27
	Spikelet Fertility (%)	Control	17.71	13.3	10.63	12.27
		Heat Stress	78.17	88.52	62.68	40.22

12. Pusa Rice Restorer 402 (PRR 402) (IC0637551; INGR21012), a Rice (*Oryza sativa* var. *indica*) Germplasm Tropical japonica based NPT line, which is a restorer of WA cytoplasm possessing the restorer gene, *Rf4*, developed in the background of a popular indica rice variety Pusa 44.

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Rice is grown in an area of about 44 million hectares in India with an annual production of about 105 million tonnes of milled rice and average productivity of 2,500 kg of paddy per ha. But the present trend of rice production and productivity will be inadequate to meet the future demand of the fast-growing Indian population. Therefore adoption of hybrid rice is one of the alternate tools to mitigate the yield barrier. Hybrid rice technology in India so far was exclusively dependent on the *indica* germplasm but the level of heterosis is low. Parental lines of the *indica* background are relatively less diverse compared to *japonica* types. Among the various available rice germplasm lines, Tropical *japonica* (TRJ) rice germplasm enjoys special reference as an alternative source for increasing heterosis level in rice. While introducing the new plant type (NPT) concept at the International Rice Research Institute, Philippines (IRRI), Khush (1995) suggested that the ideotype approach is effective for breaking the yield ceiling of an irrigated rice crop.

Keeping this in mind, a series of cross combinations were made between Pusa 44, a popular non-aromatic rice variety released from ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi with diverse TRJ lines sourced from

IRRI. The pedigree breeding approach was followed to develop NPT lines. Selection was focussed primarily on NPT characters. A total of 310 lines possessing desirable NPT traits were identified through an evaluation conducted at New Delhi in the ICAR-IARI research farm. The selected lines were further screened by molecular markers namely DRRM-RF3-5, DRRM-Rf3-10 and RMS-SF21-5 linked with fertility restorer genes *Rf3* and RMS-SF21-5 and RM 6100 linked with *Rf4*. Based on marker data 42 restorers were shortlisted and field evaluated during *Kharif* 2017 at three diverse locations viz., New Delhi, Gerua (Assam) and Aduthurai (Tamil Nadu). The restorers were also crossed to a WA-CMS line "Pusa 6A", to understand their restoration ability and evaluated at the aforesaid 3 different aforesaid locations.

Morpho-agronomic characteristics: Based on the multilocation evaluation Pusa Rice Restorer 402 (PRR 402), a tropical *japonica* based NPT line, which is a restorer of WA cytoplasm possessing the restorer gene *Rf4* (Shidenur *et al* 2019), developed in the background of a popular *indica* rice variety Pusa 44 has been identified as one of the promising

line. It has all NPT characters such as plant height of 85–92 cm, 10 to 13 productive tillers, sparse unproductive tillers, sturdy culm, heavy panicles having 200 to 250 grains, dark green leaves, and growth duration of 110 to 130 days. The potential of PRR402 as an efficient NPT based rice restorer for exploitation of heterosis has also been demonstrated (Shidenur *et al.* 2020). It produced fertile hybrid (pollen fertility- 91.44% to 96.46% and spikelet fertility- 80.07% to 85.32%) when crossed with a male sterile line, Pusa 6A (Table 1).

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Table 1: Level of heterosis of PRR 402 derived hybrid over the three locations

Combination	New Delhi		Aduturai		Assam		Across location	
	SH (%)	AH (%)	SH (%)	AH (%)	SH (%)	AH (%)	SH (%)	AH (%)
Pusa6A/PRR402	49.23**	68.67**	15.72*	32.63*	14.49**	26.87*	26.89**	43.12**

*P<0.05 and **P<0.01; SH: Standard heterosis (for yield per plant); AH: Average heterosis (for yield per plant)

13. Improved White ponni (IWP)-Saltol (IC0638602; INGR21013), a Rice (*Oryza sativa* var. indica) Germplasm Salinity Tolerant line.

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Salinity is one of the major abiotic stresses limiting rice productivity under marginal environments. The progress in development of salinity tolerant rice through Conventional breeding is slow due to complex nature of tolerance mechanisms. In the present study, efforts have been taken to generate NILs of a popular rice variety Improved White Ponni exhibiting increased tolerance against salinity through marker assisted introgression of a major effect QTL 'Saltol' from FL478. The hybridization was between these two parents during Kharif 2013. Three backcrosses were performed by using foreground, recombinant and background markers. Genotyping and phenotyping of BC3F1 progenies resulted in the identification of elite NILs of IWP harboring Saltol loci and with >90% of recurrent parent genome recovery. IWP-Sal tol NILs viz., 5-35, 5-36 and 5-45 exhibited enhanced tolerance against salinity under hydroponic conditions. The selected NILs were screened under field conditions for their agronomic traits. NIL # 5-36 exhibited superior agronomic performance (58% increased yield over IWP under saline conditions) and superior grain/cooking quality traits than that of IWP. Evaluation of NILs for grain quality traits showed that NILs were found to possess comparable grain quality and cooking quality traits as that of Improved White Ponni. All the three NILs found to possess

slender grain type with L/B ratio ranging between 2.65 and 2.86. Overall this study led to the development of salinity tolerant versions of Improved White Ponni through Marker Assisted Back Cross Breeding. (Valarmathi *et al.*, 2019). Under AICRP, IWP- Sal tol was tested under moderate coastal saline tolerant variety trial (CSTVT) at three locations namely Goa (GOA), Kerala (KE) and AP (Andhra Pradesh). Results revealed that IWP-Saltol recorded 1108 kg/ha under salinity stress which is 57.6% over recurrent parent IWP (703 kg/ha). Pooled analysis of data under Multi Location Trials conducted in various locations of Tamil Nadu revealed that IWP-Sal tol recorded 3595 kg/ha which is 16.6% over IWP (RP) (3111 kg/ha). Newly developed salinity tolerant version of Improved white ponni will help in sustaining rice yield under salinity prone areas and it will serve as ideal genetic material for functional genomic studies.

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14. Kolajoha (IC00298323; INGR21014), a Rice (*Oryza sativa* var. *indica*) Germplasm Salinity tolerant.

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Kolajoha is a popular small grain non-Basmati type aromatic rice genotype cultivated in the North-Eastern state of Assam, India. It is selected for its notable aroma in grain and its salinity tolerant status. It has given the GI tagged due to its unique aroma.

Morpho-agronomic characteristics: The genotype is diploid with awnless seed. Seeds are having black coat colour and yellowish white superfine kernel. Kernel length is < 5.5 mm and length to breadth ratio is <3. Grains have strong aroma with 100-200% kernel elongation after cooking. It is tall, photosensitive, takes long duration to mature (160 days).]

Associated characters and cultivation practices: The genotype is low yielding but preferred for preparing table

rice, Kheer, Pulao and frumenty. The most commonly present compound in Kola Joha is 2-acetyl-1- pyrroline which is responsible for its aroma. It resulted in lower SES, Na⁺ concentration, ratio of Na⁺/ K⁺ and higher K⁺ content under 100 mM salt (NaCl) stress at seedling stage which shows it is highly salinity tolerant. It is adapted to local conditions in Assam where Basmati cannot be grown.

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15. Negheri bao 1 (IC0394535; INGR21015), a Rice (*Oryza sativa*) Germplasm Anaerobic germination tolerant.

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Negheri bao is the indigenous landrace variety collected from North Lakhimpur of Assam. It is selected for its ability to germinate and survive under anaerobic conditions. The SNP genotyping of this landraces has been done by using 50K SNP rice genic chip (Rohilla et. al. 2020).

Morpho-agronomic characteristics: Negheri bao 1 is diploid rice plant (2n=24) which underflooding conditions can grow up to a height of 2 meter. It has a large sized leaf, longer panicle, and a large number of spikelets per panicle compared to crops grown without deep water situation. The culm or stem of this bao does not grow entirely erect, rather it becomes zigzag under deep-water condition. Branching does not take place under water. Adventitious roots first develop from the uppermost nodes below the water surface to extract nutrients from the water.

Associated characters and cultivation practices: The crude protein content of this brown rice is significantly high thus it is important from nutritional point of view (Mudoi and Das, 2018). The Sowing of this variety in Assam is done during March to April, as they take more than 300 days to mature. Seeds are normally sown directly in the soil before the rainy season and harvesting is done after the flood water recedes. Chemical fertilizers are not used for cultivation of this rice.

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16. Saragphala-2 (IC0591486; INGR21016), a Rice (*Oryza sativa*) Germplasm Anaerobic germination tolerant.

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Saragphala-2 is a bao rice which is indigenous landrace variety collected from North Lakhimpur of Assam. It is selected for its ability to germinate and survive under anaerobic conditions. The SNP genotyping of this landraces has been done by using 50K SNP rice genic chip (Rohilla *et al.* 2020).

Morpho-agronomic characteristics: Saragphala bao is diploid rice plant (2n=24) which under flooding condition can grow up to a height of more than 1 meter. It has a large sized leaf, longer panicle, and a large number of spikelets per panicle compared to crops grown without deep water situation. The culm or stem of this bao does not grow entirely erect, rather it becomes zigzag under deep-water condition. Branching does not take place under water. Adventitious roots first develop from the uppermost nodes below the water surface to extract nutrients from the water.

Associated characters and cultivation practices: Under submergence stress conditions at germination stage, this

genotype have ability to get enough oxygen for their metabolic activity and rapid coleoptile elongation ability. It is anaerobic germination tolerant genotype also having high anaerobic index than national positive check (Nanhi and Kalongchi). Its anaerobic germination tolerance ability makes this genotype very useful in direct seed sowing cultivation (Rohilla *et al.* 2020). The Sowing of this variety in Assam is done during March to April, as they take more than 300 days to mature. Seeds are normally sown directly in the soil before the rainy season and harvesting is done after the flood water recedes. Chemical fertilizers are not used for cultivation of this rice.

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17. BRW3806 (IC0637552; INGR21017), a Wheat (*Triticum aestivum*) Germplasm Resistant to wheat blast disease.

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Wheat is an important food crop in India often seriously affected by biotic and abiotic constraints causing serious yield losses. Additionally, the incidence of wheat blast in Bangladesh poses a threat to the wheat crop in the tropical and subtropical regions of Asia in sustaining food security. Therefore breeding wheat for tolerance to biotic and abiotic stresses has been the main breeding objective in any breeding programme. Genotype BRW 3806 was developed at ICAR-IIWBR, Karnal from the cross NI 5439/MACS 2496 following pureline breeding method and later on was selected by BAU-Sabour for testing in the Restricted irrigation trials under AICRP on wheat and Barley for three consecutive years from 2016-17 to 2018-19. Genotype BRW 3806 was included in Indian material sent for screening against wheat blast disease and was identified to be resistant

to wheat blast disease at Jessore, Bangladesh and Bolivia under artificial screening. BRW 3806 was also found to be resistant to all the races of *Puccinia graminis tritici* tested at seedling stage having gene combination Sr28+5+ against stem rust. This genotype carries Yr2 against yellow rust and Lr13+1+ against leaf rust.

Besides this, the genotype has shown superior performance (51.6 q/ha) under restricted irrigation condition as compared to the checks WH 1080 (45.3 q/ha), PBW 644 (45.32 q/ha) and HD 3043 (43.41 q/ha), WH 1142 (47.9 q/ha) and HD 3237 (50.0 q/ha) (~14 locations in NWPZ) (Table 1; pooled over three years). This genotype flowers and matures in about 105 days and 151 days, respectively. This genotypes had a plant height of 110cm and thousand grain weight of 43g.

Table 1: Summary of yield data for BRW 3806 along with checks in the coordinated trials.

Item	Year	Sites	Genetic stock BRW 3806	Check varieties				
				WH1080	PBW644	HD 3042	WH1142	HD 3237
Meanyield (q/ha)	2016-17	8	56.5	-	-	-	51.6	-
	2017-18	13	48.5	44.8	44.7	41.7	45.5	-
	2018-19	14	49.8	45.7	45.9	45.0	47.9	50.0
	Mean		51.60	45.25	45.3	43.35	48.33	50.0

Table 2: Yield performance of BRW 3806 in the NWPZ- RIR-TS-TAS trial during 2018-19.

Variety	Zero irrigation	One irrigation	Two irrigation	Mean Yield
BRW 3806	41.85	49.06	54.39	48.43
Checks				
WH1142	40.46	48.2	52.10	46.92
HD 3043	37.32	44.01	49.25	43.53
PBW 644	38.72	48.2	52.10	44.54
WH 1080	39.06	46.66	50.82	45.10

During 2018-19, this genotype was also evaluated in the agronomy trial (NWPZ- RIR-TS- TAS) and BRW 3806 (48.43 q/ha) top ranked among all the test and check varieties (ten locations) (Table 2). BRW 3806 has shown good chapatti making quality (7.60) and biscuit making quality (7.79) with the gluten index of 95.

The resistant to wheat blast disease and superior performance under restricted irrigation conditions makes BRW 3806, a potential donor to be utilized for breeding wheat for blast resistance and moisture tolerance.

18. ER9-700 (IC0637553; INGR21018), a Wheat (*Triticum aestivum*) Germplasm with Novel Leaf Rust Resistance introgressed from *Aegilops markgrafii*.

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Plant germplasm registration of ER9-700, a *T. aestivum* genetic stock carrying *Ae. markgrafii* introgression. ER9-700 is highly resistant to leaf rust pathogen *Puccinia triticina* and the resistance gene (*LrM*) is mapped to telomeric end of short arm of 2A chromosome. *LrM* can be utilized for imparting disease resistance in wheat breeding programmes.

ER9-700 is highly resistant to leaf rust (*Puccinia triticina* Eriks.) showing infection types "0" or "0;" against 15 leaf rust races. It was developed with the intent of transferring novel gene(s) from C genome of *Aegilops markgrafii* (CC; $2n=2x=14$) to bread wheat at Division of Genetics, IARI, New Delhi. The 5B nullisomy method was used to induce homoeologous pairing and recombination between wheat and *Ae. markgrafii* chromosomes (Riley and Chapman 1958; Sears and Okamoto 1958). A 5B monosomic plant (mono 5B, $2n - 1 = 41$) in bread wheat cultivar Lal Bahadur was emasculated and pollinated using *Ae. markgrafii* (Accession No. EC331770, PI 369571) pollen. The interspecific F_1 plants with 27 chromosomes were cytologically identified at metaphase I of meiosis following the method described earlier (Niranjana *et al.* 2017) and were backcrossed using pollen of Lal Bahadur ($2n=6x=42$). Leaf rust resistant BC_1F_1 plants were backcrossed with Lal Bahadur and selfed to develop BC_2F_5 generation. One of the lines ($BC_2F_{4.5}$) viz.,

ER-9 was homozygous for leaf rust resistance showing 3:1 (resistant: susceptible) segregation. But it exhibited sterility in a few spikelets. Hence, fresh pollen from a few individual plants of ER-9 (BC_2F_9 generation) were used to pollinate Agra Local. One of the F_2 populations showed complete fertility and segregated into 3 resistant: 1 susceptible, was advanced to the next generation and maintained by self-pollination. One of the resistant F_4 plants showed normal 21 bivalent configurations in all the PMCs which was named 'ER9-700'. Genetic analysis revealed that a single dominant gene (*LrM*) imparts leaf rust resistance in ER9-700. This gene was mapped to short arm of chromosome 2A using two SSR and three SNP-based PCR markers. Three SNP based PCR markers viz., SNP_AX-948171722AS, SNP_AX-945380402AS and SNP_AX-945219402AS were closest at a distance of 2.0 cM from *LrM*. Genomic in situ hybridization confirmed the presence of C genome toward the telomeric end of a pair of chromosomes (Kirti *et al.* 2020). Several leaf rust resistance genes have been derived from wild species, however, there is no report of introgression of leaf rust resistance genes from *Ae. markgrafii* (McIntosh *et al.* 2017). This novel leaf rust resistance gene (*LrM*) will diversify the repertoire of rust resistance genes useful in wheat rust resistance breeding.

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19. TMD6-4 (IC0637554; INGR21019), a Wheat (*Triticum aestivum*) Germplasm with Leaf Rust Resistance from *Triticum militinae* introgression.

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Plant germplasm registration of TMD6-4, *T. aestivum* genetic stock carrying *T. militinae* introgression. TMD6-4 exhibited resistance to leaf rust pathogen *Puccinia triticina* Eriks. and exhibits infection types ranging from '0;' to '1+' against fifteen pathotypes from five diverse groups and can be utilized for broadening the genetic base and rust resistance in wheat breeding programmes.

T. militinae Zhuk. et Migusch. (A⁺G, 2n=4x=28) is a tetraploid wild species belonging to the secondary gene pool of wheat. It is a free-threshing form of *T. timopheevii* and was discovered by Zhukovsky in 1950 (Zhukovsky and Migushova 1969; Jakobson et al. 2006). A *T. militinae* accession (117001) was received from Institute of Plant Science Research, Norwich, England in 1993. TMD6-4 is derived from the cross CS/*T. militinae*//3*NI5439. Meiotic analysis of TMD6-4 showed 21 bivalents indicating its cytological stability (Nataraj et al. 2018). TMD6-4 exhibited high degree of seedling stage resistance with infection type (IT) ranging from '0;' to '1+' against fifteen pathotypes from five diverse groups (12, 77, 104, 108, 162) indicating broad-spectrum resistance towards leaf rust. Further, to identify the *T. militinae* introgressions in TMD6-4, molecular characterization was carried out using SSR markers (Nataraj et al. 2018). Results indicated that TMD6-4 carried 2.8% of introgression from *T. militinae*. Genome wise analysis of

introgression in TMD6-4 showed maximum introgression (3.7%) in B genome, 2.5% in A genome and 2.2% in D genome. The pairing of chromosomes during meiosis between "A" of Chinese Spring and "A⁺" of *T. militinae* is primarily homologous, while B and G genomes are similar and shows homoeologous pairing (Gill and Chen 1987). Hence transfer of genes from both A⁺ and G genomes of *T. militinae* is possible. TMD6-4 is a good source of novel leaf rust resistance and can be used as donor for imparting leaf rust resistance in wheat improvement programme.

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20. TMD 11-5 (IC0637555; INGR21020), a Wheat (*Triticum aestivum*) Germplasm with Leaf Rust Resistance from *Triticum militinae* introgression.

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Plant germplasm registration of TMD11-5, a *T. aestivum* genetic stock carrying *T. militinae* introgressions. TMD11-5 is highly resistant to leaf rust pathogen *Puccinia tritica* and exhibits infection types ranging from '0;' to '1+' against fifteen pathotypes from five diverse groups. The resistance can be utilized for imparting disease resistance in wheat breeding programmes.

TMD11-5 is a genetic stock derived from the cross CS/T. *militinae* acc. no. 117001/3*CS and was found to be cytologically stable (Nataraj *et al.* 2018). *T. militinae* Zhuk. et Migusch. (A¹G, 2n=4x=28) belongs to the secondary gene pool and is a free-threshing form of *T. timopheevii* which was discovered by Zhukovsky in 1950 (Zhukovsky and Migushova 1969; Jakobson *et al.* 2006). Some consider it as a spontaneous mutant of *T. timopheevii* (Dorofeev *et al.* 1987) and others believe it to have originated from an introgressive hybridization between *T. timopheevii* and *T. carthlicum* Nevski (*T. persicum* Vav.) (Navruzbekov 1981; Jarve *et al.* 2002). *T. militinae* acc. no. 117001 was received in 1993 from Institute of Plant Science Research, Norwich, Norfolk, England, Great Britain. TMD11-5 showed high degree of seedling stage resistance with infection type (IT) ranging from '0;' to '1+' against fifteen pathotypes from five diverse groups viz., 12, 77, 104, 108, 162. Analysis of SSR genotyping data confirmed the introgression of 8.6% genomic regions from *T. militinae* into TMD11-5 (Nataraj *et al.* 2018). TMD11-5 showed maximum introgression of 12.2% in A genome, 6.8% in B genome, 6.8% in D genome. Since B and G genome share greater homology between them than with any other

genome of the Triticeae (Gill and Chen 1987), gene transfer from timopheevii wheat to common wheat is possible by direct hybridization. The pairing of chromosomes during meiosis between A and A¹ genomes is primarily homologous while pairing between B and G genomes is homoeologous (Gill and Chen 1987) enabling transfer of genes from both A¹ and G genomes of timopheevii wheat.

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21. DWAP 1608 (IC0638605; INGR21021), a Wheat (*Triticum aestivum*) Germplasm with Heat and Drought Tolerance.

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The wheat growing areas in the country are exposed to several abiotic stresses during crop growth stages in India. Among these, heat stress becoming important which affects grain filling and thereby lowering the yield potential of the genotypes. Simultaneously the wheat areas in central and peninsular India are experiencing water deficit conditions resulting in drought conditions. Keeping in view a systematic research effort as wheat improvement for warmer areas was initiated at ICAR-IIWBR, Karnal with focus on development of early maturing wheat genotypes that can withstand

terminal heat stress during grain growth period and yield more than the other traditional cultivars. This programme has major component of shuttle breeding approach that resulted in development of heat stress as well as drought stress tolerant varieties. In this programme, a number of advanced genotypes were developed and contributed to yield trials and nurseries.

The genetic stock DWAP 1608 (28 ESWYT 107/RAJ 4037) was developed through modified pedigree method. The female parental line 28ESWYT107 (BL1496/Milan/3/Croc_1/

Performance of entries in multi-locational station trial under late sown condition

Entry	Yield (q ha ⁻¹)	Leaf rust (ACI)	Black rust (ACI)
DWAP 1608	41.9	5S (1.7)	20MS (8.2)
DBW 71 (C)	39.6	5S (1.0)	20MS (7.0)
DBW 14 (C)	37.8	10S (3.2)	60S (22.7)

Ae. squarrosa (205)//Kauz) was germplasm line selected from CIMMYT nursery in 2007-08. The male parental line Raj 4037 was high yielding variety released for timely sown irrigated conditions of Peninsular zone (PZ). As PZ is the only zone where crop exposed to early heat, terminal heat stress in addition to drought conditions, this cross was made to develop heat as well as drought tolerant genotype. DWAP 1608 was evaluated in IIWBR station trial 3 meant for late sown irrigated conditions. Based on its superior performance to the checks, it was promoted in NIVT 3B as DBW 271 during 2017-18 crop season.

At the same time, this genotype was contributed in multilocal trial NICRA trials and was evaluated for two consecutive crop seasons 2017-18 and 2018-19. The trial was conducted under different production conditions namely timely sown irrigated, timely sown rainfed, late sown irrigated and late sown rainfed conditions at Akola, Karnal and Powarkheda alongwith six heat and drought tolerant check genotypes. Simultaneously same set was

also evaluated under rain out shelter during both the crop seasons at Karnal. Data on yield components and phenological traits were recorded under all the three conditions. Susceptibility index was calculated considering irrigated timely sown as control or non-stressed condition, timely rainfed as drought stress, irrigated late sown as heat stress and rainfed late as heat and drought stress together. Based on pooled data of two crop seasons across three locations namely Akola of PZ, Powarkheda of CZ and Karnal in NWPZ under late sown irrigated conditions, DWAP 1608 was identified as heat tolerant genotype (Sareen *et al.*, 2019). Similarly, pooled data under late sown rainfed condition also categorized DWAP 1608 as drought and heat tolerant genotype.

It is concluded that the genotype DWAP 1608 is highly tolerant to heat as well as drought stress conditions which may be a potential donor in future wheat improvement programmes for tolerance to abiotic stress conditions.

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Pooled susceptibility index of promising entries in multilocation trial during 2017-18 & 2018-19

Genotype	Akola (PZ)	Powarkheda (CZ)	Karnal (NWPZ)	Pooled
Heat susceptibility index (HSI) under late sown irrigated conditions				
DWAP 1608	2.74	-1.29	1.01	0.42
AKW 2862-1 (C)	1.72	0.61	0.87	0.74
HTW 11 (C)	1.55	0.00	0.97	0.81
HINDI 62 (C)	-0.71	2.19	0.53	0.86
DHTW 60 (C)	0.77	2.17	0.52	0.90
Drought & heat susceptibility index (DHSI) under late sown rainfed conditions				
DWAP 1608	1.00	0.12	0.87	0.77
AKW 3717 (C)	0.70	1.05	0.84	0.85
HINDI 62 (C)	0.59	1.37	0.85	0.94
HTW 11 (C)	0.97	0.60	1.10	0.98
HTW 6 (C)	0.86	1.01	1.07	1.00

22. KHTW-1 (BST1 (ST 1A) (IC0637557; INGR21022), a Wheat (*Triticum aestivum* subsp. *aestivum*) Germplasm with Heat Tolerance and Better Heat Susceptibility Index.

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As global climate change is becoming evident, the staple food crops such as wheat are facing the brunt of increased events of high temperature stress. High temperature stress especially at grain filling duration is affecting wheat yield significantly. Identification and development of heat tolerant wheat germplasm lines is therefore of prime importance nowadays. In this direction under the NICRA project, 42 wheat germplasm lines along with 4 heat tolerant checks were screened for heat tolerance under timely and late sown conditions.

The experiment was conducted at three locations viz., Akola, Powarkheda and Karnal during two years i.e. 2017-18 and 2018-19. Grain yield and related phenological parameters were recorded. Heat susceptibility index (HSI)

was calculated considering timely sown irrigated condition as control. Based on the mean HSI over years and locations it was found that, BST1(ST 1A) was having significantly lower HSI values than that of the checks.

BST1 (ST 1A) found to be superior with HSI of 0.24 in comparison to all the four heat tolerant check varieties viz., AKW2862-1 (HSI=0.74), HTW11 (HSI=0.81), Hindi 62 (HSI=0.86) and HTW60 (HSI=0.9). BST1 (ST 1A) was also superior to all the test genotypes. Therefore, BST1 (ST 1A) would be a potential source as heat tolerance donor parent. This valuable germplasm may be utilized in breeding programs to develop bread wheat varieties suitable for terminal heat tolerance.

Table 1: Heat susceptibility index (HSI) of genotypes tolerant to heat stress under late sown irrigated conditions (pooled over three locations and two crop seasons)

Genotypes	Karnal	Powarkheda	Akola	Pooled
KHTW-1 (BST1 (ST 1A))	0.42	0.24	1.91	0.24
QBP 1606	0.56	-0.17	-1.41	0.27
HTW 65	0.86	0.81	0.16	0.35
DWAP 1608	1.01	-1.29	2.74	0.42
HTW 67	1.04	-0.53	0.72	0.46
WS 2016-12	0.64	0.12	2.42	0.50
CNM 16-Jan	0.3	0.88	1.52	0.53
QBP 1605	0.5	0.54	1.63	0.53
QBP 1608	1	-0.18	1.21	0.58
WS 2016-4	0.6	0.57	0.14	0.59
HTW 64	0.8	0.8	-1.03	0.62
DWAP 1607	1.08	-0.09	0.39	0.70
RWP 2016-10	0.84	0.91	2.6	0.79
LBP 2016-9	1.18	0.82	-0.81	0.96
AKW 2862-1 (C)	0.87	0.61	1.72	0.74
HTW 11 (C)	0.97	0	1.55	0.81
HINDI 62 (C)	0.53	2.19	-0.71	0.86
DHTW 60 (C)	0.52	2.17	0.77	0.9

23. DBW 243 (IC0637558; INGR21023), a Wheat (*Triticum aestivum*) Germplasm High Water Use Efficiency.

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India is the second largest wheat producer in the world and it cultivated in an area of about 30 mha (GOI, 2017). The assured supplemental irrigation is major factor during rabi season helps in harvesting the potential of the crop. During the recent decades it has been experienced a steady increase in the depth of the groundwater table in wheat

growing regions of Indo Gangetic plains (Hira, 2009; Rodell *et al.*, 2009). The current water productivity of wheat is estimated to be about 0.8 - 1.06 kg m⁻³ in India (Meena *et al.*, 2015; Zwart *et al.*, 2010). Uncontrolled irrigation supported by subsidized/free electricity clubbed with low water productivity is leading to unsustainability of the traditional

wheat cultivation (Humphreys *et al.*, 2010; Meena *et al.*, 2019). The situation therefore calls for development of improved agronomic management options as well as identification of water use efficient genotypes with better yield. Looking at the necessity for wheat genotypes with high water productivity which can be readily adoptable at farmer's field, the present study has been undertaken aiming at identifying high WUE wheat genotypes under limited moisture conditions. Seventy-one genetically diverse wheat

genotypes were screened for high water use efficiency (WUE) under limited soil moisture level at 60 % of Cumulative Pan Evaporation (CPE) during 2015-16. Out of these best performing sixteen high WUE genotypes were shortlisted for a detailed field study during 2016-17 and 2017-18. Tukey's test of significance led ranking and GGE biplot analysis identified DBW243 as high water use efficient genotype with a water use efficiency of 2.40 kg m⁻³ at 60% CPE over check variety HD2967 (1.99 kg m⁻³) during two years of testing.

Table 1: Average Water Use Efficiency of wheat genotype DBW243 in comparison on the basis of experiment during 2016-17 and 2017-18

Genotypes	WUE 60%			WUE 80%			WUE Avg
	16-17	17-18	Avg	16-17	17-18	Avg.	
	kg m ⁻³			kg m ⁻³			kg m ⁻³
DBW243	2.85	1.94	2.40	2.25	1.62	1.94	2.17
HD2967 (C)	2.19	1.78	1.99	2.15	1.42	1.79	1.89
PBW 550 (C)	2.03	1.43	1.73	1.65	1.26	1.46	1.59
WH1105 (C)	2.22	1.69	1.95	1.91	1.54	1.72	1.84
DBW 88 (C)	2.42	1.69	2.06	2.00	1.59	1.79	1.93
LSD at P< 0.05	0.34	0.19		0.18	0.25		

Table 2: Average Wheat grain yield, of wheat genotype DBW243 in comparison on the basis of experiment during 2016-17 and 2017-18

Genotypes	GY at 60% CPE		GY at 80% CPE		Avg GY
	16-17	17-18	16-17	17-18	
	kg ha ⁻¹		kg ha ⁻¹		
DBW243	5074	4874	5326	4968	5060
HD2967 (C)	3899	4467	5104	4350	4455
PBW 550 (C)	3619	3579	3919	3875	3748
WH1105 (C)	3945	4241	4519	4719	4356
DBW 88 (C)	4315	4255	4731	4869	4542
LSD at P< 0.05	607	486	438	762.6	

24. DCMS 17A & DCMS 17B (IC0637559 & IC0637560; INGR21024), a New CMS (A) Line of Wheat (*Triticum aestivum*) in DBW17 Background with CMS Source (Chuan 18A) along with Maintainer (B) Line.

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Exploitation of heterosis and development of hybrids is an innovative approach in cereals for breaking yield barriers and realizing higher yields. Indian hybrid wheat programme has re- oriented in 1995 with introduction of some cytoplasmic male sterile (CMS) lines from CIMMYT, Mexico but these could not be utilized due to their un-adapted agronomic background. In 2005, the diversification of CMS lines received from CIMMYT was initiated with the objective to develop new CMS lines in the agronomic background of Indian wheat varieties so that these can be further utilized in hybrid wheat development programme. The basic *Triticum timopheevii*

based cytoplasm sources were MTSA 2A, Chuan 13A and Chuan 18A which were transferred in the background of diverse CIMMYT advance lines. These CMS lines from CIMMYT were introduced in India as source of cytoplasmic male sterility for hybrid wheat development programme.

The genetic stock DCMS 17A was developed using CHUAN 18A based CMS line CMS21A (CHUAN18A/6/7*KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ) as female parent in first cross with Indian advanced variety DBW 17 as male parent. DBW 17 was a landmark variety for north western plains zone as well as north eastern plains zone for timely sown irrigated

condition. After initial cross, 8 generations of backcrosses were made with DBW 17 as recurrent parent in order to recover its agronomic background. In this process, more than 100 spikes were pollinated. In every generation, five spikes of recipient population were bagged just after emergence from the flag leaf in order to ensure complete male sterility as indicated by no seed set.

After eight backcross generations, the resultant male sterile line was planted in the field along with recurrent parent as maintainer line. The newly developed CMS lines were pollinated with maintainer lines for getting seeds for next generation. For the purpose, the CMS line was planted along with maintainer line in 2B: 4A: 2B row ratio and all the recommended agronomic practices were adopted to raise a good crop during four consecutive crop seasons of 2015-16, 2016-17, 2017-18 and 2018-19. Five randomly selected spikes of these CMS lines were bagged at just after emergence from flag leaf and the seed set was observed in these un-pollinated spikes for calculating seed set percentage. The data were also recorded on days to heading and plant height for evaluating their suitability to hybrid wheat programme. The seed set was observed among these lines and the results indicated no seed set in the bagged un-pollinated spikes of the CMS lines. This indicated complete male sterility in the new CMS line DCMS 17A (Table 1).

In hybrid development programme based on three-line system, the maintenance of CMS lines is very crucial. The similarity in days to heading and plant height in CMS and maintainer lines is needed to facilitate pollination and get maximum seed set in the CMS line. The heading period in DCMS 17A ranged from 92-103 days with mean of 96 days whereas average plant height was 90 cm with range of

Table 2: Performance of DCMS 17A and its maintainer DCMS 17B for morphological traits

Traits	DCMS 17A	DCMS 17B (DBW 17)
Coleoptile Colour	Absent	Absent
Growth habit	Semi erect	Semi erect
Foliage Colour	Green	Green
Spike colour	white	white
Spike shape	Tapering	Tapering
Awn colour	White	White
Grain colour	Amber	Amber
Spike length (cm)	9	10
Spikelet number	20	22
Days to maturity	143	144

84-96 cm. Compared to this, the maintainer line DCMS 17B showed average days to heading of 97 days and plant height of 90 cm which is in tune to CMS line. This similar feature is helpful in synchronised flowering and pollen movement for maximum seed set.

In addition, few additional agro-morphological traits namely coleoptile colour, growth habit, foliage colour, awn colour, spike colour and shape and grain colour were observed over these years which showed similar pattern to the parental maintainer line DBW 17 (Table 2). The results also indicated similarity in spikelet number per spike, spike length and maturity period in DCMS 17A as compared to the maintainer line.

It may be concluded that the genetic stock DCMS 17A may be used intensively for development of hybrid wheat for Indian conditions so that new breakthrough may be achieved in wheat productivity.

Table 1: Performance of DCMS 17A and its maintainer for quantitative traits

Year	DCMS17A (CMS- A line)			DCMS 17B (DBW 17: B line)	
	Male sterility(%)	Days to heading	Plant height(cm)	Days to heading	Plant height(cm)
2015-16	100	97	86	97	88
2016-17	100	93	84	93	90
2017-18	100	92	96	94	87
2018-19	100	103	94	102	94
Mean	100	96	90	97	90

25. DCMS 24A & DCMS 24B (IC0637561 & IC0637562; INGR21025), a New CMS (A) line of Wheat (*Triticum aestivum*) in DBW 16 Background with CMS Source (Chuan 18A) along with Maintainer (B) Line

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Exploitation of heterosis and development of hybrids is an innovative approach in cereals for breaking yield barriers and realizing higher yields. Indian hybrid wheat programme has re- oriented in 1995 with introduction of some cytoplasmic male sterile (CMS) lines from CIMMYT, Mexico but these could not be utilized due to their un-adapted agronomic background. In 2005, the diversification of CMS lines received from CIMMYT was initiated with the objective to develop new CMS lines in the agronomic background of Indian wheat varieties so that these can be further utilized in hybrid wheat development programme. The basic *Triticum timopheevii* based cytoplasm sources were MTSA 2A, Chuan 13A and Chuan 18A which were transferred in the background of diverse CIMMYT advance lines. These CMS lines from CIMMYT were introduced in India as source of cytoplasmic male sterility for hybrid wheat development programme.

The genetic stock DCMS 24A was developed using CHUAN 18A based CMS line CMS 10A (CHUAN 18A/CHUAN 18B//7*KAUZ/HEVO) as female parent in first cross with Indian advanced variety DBW 16 as male parent. DBW 16 was a high yielding variety for north western plains zone for late sown irrigated condition. After initial cross, 8 generations of backcrosses were made with DBW 16 as recurrent parent in order to recover its agronomic background. In this process, more than 100 spikes were pollinated. In every generation, five spikes of recipient population were bagged just after emergence from the flag leaf in order to ensure complete male sterility as indicated by no seed set.

After eight backcross generations, the resultant male sterile line was planted in the field along with recurrent parent as maintainer line. The newly developed CMS lines were pollinated with maintainer lines for getting seeds for next generation. For the purpose, the CMS line was planted along with maintainer line in 2B: 4A: 2B row ratio and all the recommended agronomic practices were adopted to raise a good crop during four consecutive crop seasons of 2015-16, 2016-17, 2017-18 and 2018-19. Five randomly selected spikes of these CMS lines were bagged at just after emergence from flag leaf and the seed set was observed in these un-pollinated spikes for calculating seed set percentage. The data were also recorded on days to heading and plant height for evaluating their suitability to hybrid wheat programme. The seed set was observed among these lines and the results indicated no seed set in the bagged un-pollinated spikes of

the CMS lines. This indicated complete male sterility in the new CMS line DCMS 24A (Table 1).

In hybrid development programme based on three-line system, the maintenance of CMS lines is very crucial. The similarity in days to heading and plant height in CMS and maintainer lines is needed to facilitate pollination and get maximum seed set in the CMS line. The heading period in DCMS 24A ranged from 95-108 days with mean of 100 days whereas average plant height was 98 cm with range of 91-102 cm. Compared to this, the maintainer line DCMS 24B showed average days to heading of 100 days and plant height of 97 cm which is in tune to CMS line. This similar feature is helpful in synchronised flowering and pollen movement for maximum seed set.

In addition, few additional agro-morphological traits namely coleoptile colour, growth habit, foliage colour, awn colour, spike colour and shape and grain colour were observed over these years which showed similar pattern to the parental maintainer line DBW 16 (Table 2). The results also indicated similarity in spikelet number per spike, spike length and maturity period in DCMS 24A as compared to the maintainer line DBW 16.

It may be concluded that the genetic stock DCMS 24A may be used intensively for development of hybrid wheat for Indian conditions so that new breakthrough may be achieved in wheat productivity.

Table 2: Performance of DCMS 24A and its maintainer DCMS 24B for agro-morphological traits

Traits	DCMS 24A	DCMS 24B (DBW 16)
Coleoptile Colour	Absent	Absent
Growth habit	Semi erect	Semi erect
Foliage Colour	Green	Green
Spike colour	white	white
Spike shape	Tapering	Tapering
Awn colour	White	White
Grain colour	Amber	Amber
Spike length (cm)	10	9
Spikelet number	19	19
Days to maturity	149	148

Table 1: Performance of DCMS24A and its maintainer for quantitative traits

Year	DCMS24A (CMS- A line)			DCMS 24B (DBW 16: B line)	
	Male sterility(%)	Days to heading	Plant height(cm)	Days to heading	Plant height(cm)
2015-16	100	99	91	101	96
2016-17	100	95	100	95	101
2017-18	100	97	98	97	97
2018-19	100	108	102	108	92
Mean	100	100	98	100	97

26. DCMS 34A and 34B (IC0637563 & IC0637564; INGR21026), a New CMS (A) Line of Wheat (*Triticum aestivum*) in PBW 502 Background with CMS Source (Chuan 18A) alongwith Maintainer (B) Line

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Exploitation of heterosis and development of hybrids is an innovative approach in cereals for breaking yield barriers and realizing higher yields. Indian hybrid wheat programme has re- oriented in 1995 with introduction of some cytoplasmic male sterile (CMS) lines from CIMMYT, Mexico but these could not be utilized due to their un-adapted agronomic background. In 2005, the diversification of CMS lines received from CIMMYT was initiated with the objective to develop new CMS lines in the agronomic background of Indian wheat varieties so that these can be further utilized in hybrid wheat development programme. The basic *Triticum Timopheevii* based cytoplasm sources were MTSA 2A, Chuan 13A and Chuan 18A which were transferred in the background of diverse CIMMYT advance lines. These CMS lines from CIMMYT were introduced in India as source of cytoplasmic male sterility for hybrid wheat development programme.

The genetic stock DCMS 34A was developed using CHUAN 18A based CMS line CMS21A (CHUAN18A/6/7*KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ) as female parent in firstcross with Indian advanced variety PBW 502 as male parent. PBW 502 was a released variety for north western plains zone for timely sown irrigated condition. After initial cross, 8 generations of backcrosses were made with PBW 502 as recurrent parent in order to recover its agronomic background. In this process, more than 100 spikes were pollinated. In every generation, five spikes of recipient population were bagged just after emergence from the flag leaf in order to ensure complete male sterility as indicated by no seed set.

After eight backcross generations, the resultant male sterile line was planted in the field along with recurrent parent as maintainer line. The newly developed CMS lines were pollinated with maintainer lines for getting seeds for next generation. For the purpose, the CMS line was planted

along with maintainer line in 2B: 4A: 2B row ratio and all the recommended agronomic practices were adopted to raise a good crop during four consecutive crop seasons of 2015-16, 2016-17, 2017-18 and 2018-19. Five randomly selected spikes of these CMS lines were bagged at just after emergence from flag leaf and the seed set was observed in these un-pollinated spikes for calculating seed set percentage. The data were also recorded on days to heading and plant height for evaluating their suitability to hybrid wheat programme. The seed set was observed among these lines and the results indicated no seed set in the bagged un-pollinated spikes of the CMS lines. This indicated complete male sterility in the new CMS line DCMS 34A (Table 1).

In hybrid development programme based on three-line system, the maintenance of CMS lines is very crucial. The similarity in days to heading and plant height in CMS and maintainer lines is needed to facilitate pollination and get maximum seed set in the CMS line. The heading period in

Table 2: Performance of DCMS 34A and its maintainer DCMS 34B for morphological traits

Traits	DCMS 34A	DCMS 34B (PBW 502)
Coleoptile Colour	Absent	Absent
Growth habit	Semi erect	Semi erect
Foliage Colour	Green	Green
Spike colour	white	white
Spike shape	Tapering	Tapering
Awn colour	White	White
Grain colour	Amber	Amber
Spike length (cm)	11	11
Spikelet number	20	20
Days to maturity	149	147

Table 1: Performance of DCMS 34A and its maintainer for quantitative traits

Year	DCMS34A (CMS- A line)			DCMS 34B (PBW 502: B line)	
	Male sterility(%)	Days to heading	Plant height(cm)	Days to heading	Plant height(cm)
2015-16	100	102	104	102	95
2016-17	100	94	98	94	96
2017-18	100	97	97	97	97
2018-19	100	105	102	106	107
Mean	100	100	100	100	99

DCMS 34A was ranged from 94-105 days with mean of 100 days whereas average plant height was 100 cm with range of 97-104 cm. Compared to this, the maintainer line DCMS 34B (PBW 502) showed average days to heading of 100 days and plant height of 99 cm which is in tune to CMS line. This similar feature is helpful in synchronised flowering and pollen movement for maximum seed set.

In addition, few additional agro-morphological traits namely coleoptile colour, growth habit, foliage colour, awn colour, spike colour and shape and grain colour were observed over these years which showed similar pattern to

the parental maintainer line PBW 502 (Table.2). The results also indicated similarity in spikelet number per spike, spike length and maturity period in DCMS 34A as compared to the maintainer line PBW 502. The maturity days was more in the DCMS 34AA which is beneficial to get good grain development in the CMS line as well as seed set while attempting hybrid combinations.

It may be concluded that the genetic stock DCMS 34A may be used intensively for development of hybrid wheat for Indian conditions so that new breakthrough may be achieved in wheat productivity.

27. DCMS 37A and 37B (IC0637565 & IC0637566; INGR21027), a New CMS Line of Wheat (*Triticum aestivum*) in DBW 55 Background With CMS Source (Chuan 18A) alongwith Maintainer (B) Line

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Exploitation of heterosis and development of hybrids is an innovative approach in cereals for breaking yield barriers and realizing higher yields. Indian hybrid wheat programme has re- oriented in 1995 with introduction of some cytoplasmic male sterile (CMS) lines from CIMMYT, Mexico but these could not be utilized due to their un-adapted agronomic background. In 2005, the diversification of CMS lines received from CIMMYT was initiated with the objective to develop new CMS lines in the agronomic background of Indian wheat varieties so that these can be further utilized in hybrid wheat development programme. The basic *Triticum Timopheevii* based cytoplasm sources were MTSA 2A, Chuan 13A and Chuan 18A which were transferred in the background of diverse CIMMYT advance lines. These CMS lines from CIMMYT were introduced in India as source of cytoplasmic male sterility for hybrid wheat development programme.

The genetic stock DCMS 37A was developed using CHUAN 18A based CMS line CMS15A (CHUAN18A//7*ATTILA/3BCN) as female parent in first cross with Indian advanced variety DBW 55 as male parent. DBW 55 was a high yielding elite line evaluated in NIVT 1A meant for north western plains zone as well as north eastern plains zone under timely sown irrigated condition. After initial cross, 8 generations of backcrosses were made

with DBW 55 as recurrent parent in order to recover its agronomic background. In this process, more than 100 spikes were pollinated. In every generation, five spikes of recipient population were bagged just after emergence from the flag leaf in order to ensure complete male sterility as indicated by no seed set.

After eight backcross generations, the resultant male sterile line was planted in the field along with recurrent parent as maintainer line. The newly developed CMS lines were pollinated with maintainer lines for getting seeds for next generation. For the purpose, the CMS line was planted along with maintainer line in 2B: 4A: 2B row ratio and all the recommended agronomic practices were adopted to raise a good crop during four consecutive crop seasons of 2015-16, 2016-17, 2017-18 and 2018-19. Five randomly selected spikes of these CMS lines were bagged at just after emergence from flag leaf and the seed set was observed in these un-pollinated spikes for calculating seed set percentage. The data were also recorded on days to heading and plant height for evaluating their suitability to hybrid wheat programme. The seed set was observed among these lines and the results indicated no seed set in the bagged un-pollinated spikes of the CMS lines. This indicated complete male sterility in the new CMS line DCMS 37A (Table 1).

Table 1: Performance of DCMS 37A and its maintainer for quantitative traits

Year	DCMS 37A (CMS- A line)			DCMS 37B (DBW 55: B line)	
	Male sterility(%)	Days to heading	Plant height(cm)	Days to heading	Plant height(cm)
2015-16	100	93	97	93	98
2016-17	100	94	95	94	100
2017-18	100	97	100	97	103
2018-19	100	104	92	104	99
Mean	100	97	96	97	100

In hybrid development programme based on three-line system, the maintenance of CMS lines is very crucial. The similarity in days to heading and plant height in CMS and maintainer lines is needed to facilitate pollination and get maximum seed set in the CMS line. The heading period in DCMS 37A was ranged from 93-104 days with mean of 97 days whereas average plant height was 96 cm with range of 92-100 cm. Compared to this, the maintainer line DCMS 37B (DBW55) showed average days to heading of 97 days and plant height of 100 cm which is in tune to CMS line. This similar feature is helpful in synchronised flowering and pollen movement for maximum seed set.

In addition, few additional agro-morphological traits namely coleoptile colour, growth habit, foliage colour, awn colour, spike colour and shape and grain colour were observed over these years which showed similar pattern to the parental maintainer line DBW 55 (Table.2). The results also indicated comparable spikelet number per spike, spike length and maturity period in DCMS 37A as compared to the maintainer line DBW 55. The maturity days was more in the DCMS37A which is beneficial to get good grain development

Table 2: Performance of DCMS 37A and its maintainer DCMS 37B for morphological traits

Traits	DCMS 37A	DCMS 37B (DBW 55)
Coleoptile Colour	Absent	Absent
Growth habit	Semi erect	Semi erect
Foliage Colour	Green	Green
Spike colour	white	white
Spike shape	Tapering	Tapering
Awn colour	White	White
Grain colour	Amber	Amber
Spike length (cm)	10	11
Spikelet number	21	23
Days to maturity	148	146

in the CMS line as well as seed set while attempting hybrid combinations.

It may be concluded that the genetic stock DCMS 37A may be used intensively for development of hybrid wheat for Indian conditions so that new breakthrough may be achieved in wheat productivity.

28. DCMS 46A and 46B (IC0637567 & IC0637568; INGR21028), a New CMS(A) Line of Wheat (*Triticum aestivum*) in CBW 38 Background with CMS Source (Chuan 18A) along with Maintainer (B) Line

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The genetic stock DCMS 46A was developed using CHUAN 18A based CMS line CMS 15A (CHUAN18A//7*ATTILA/3BCN) as female parent in first cross with Indian advanced variety CBW 38 as male parent. CBW 38 was released variety for north eastern plains zone for timely sown irrigated

condition. After initial cross, 8 generations of backcrosses were made with CBW 38 as recurrent parent in order to recover its agronomic background. In this process, more than 100 spikes were pollinated. In every generation, five spikes of recipient population were bagged just after emergence from the flag leaf in order to ensure complete male sterility as indicated by no seed set.

After eight backcross generations, the resultant male sterile line was planted in the field along with recurrent parent as maintainer line. The newly developed CMS lines were pollinated with maintainer lines for getting seeds for next generation. For the purpose, the CMS line was planted along with maintainer line in 2B: 4A: 2B row ratio and all the recommended agronomic practices were adopted to raise a good crop during four consecutive crop seasons of 2015-16, 2016-17, 2017-18 and 2018-19. Five randomly selected spikes of these CMS lines were bagged at just after emergence from flag leaf and the seed set was observed in these un-pollinated spikes for calculating seed set percentage. The data were also recorded on days to heading and plant height for evaluating their suitability to hybrid wheat programme. The seed set was observed among these lines and the results

Table 1: Performance of DCMS 46A and its maintainer for quantitative traits

Year	DCMS 46A (CMS- A line)			DCMS 46B (CBW 38: B line)	
	Male sterility (%)	Days to heading	Plant height (cm)	Days to heading	Plant height (cm)
2015-16	100	93	101	94	102
2016-17	100	94	103	94	105
2017-18	100	97	111	95	121
2018-19	100	103	104	106	111
Mean	100	97	105	97	110

Table 2: Performance of DCMS 46A and its maintainer DCMS 46B for agro-morphological traits

S.no.	Traits	DCMS 46A	DCMS 46B (CBW 38)
1	Coleoptile Colour	Absent	Absent
2	Growth habit	Semi erect	Semi erect
3	Foliage Colour	Green	Green
4	Spike colour	white	white
5	Spike shape	Tapering	Tapering
6	Awn colour	White	White
7	Grain colour	Amber	Amber
8	Spike length (cm)	9	10
9	Spikelet number	21	22
10	Days to maturity	149	147

indicated no seed set in the bagged un-pollinated spikes of the CMS lines. This indicated complete male sterility in the new CMS line DCMS 46A (Table 1).

In hybrid development programme based on three-line system, the maintenance of CMS lines is very crucial. The similarity in days to heading and plant height in CMS and maintainer lines is needed to facilitate pollination and get

maximum seed set in the CMS line. The heading period in DCMS 46A was ranged from 93-103 days with mean of 97 days whereas average plant height was 105 cm with range of 101-111 cm. Compared to this, the maintainer line DCMS 46B (CBW 38) showed average days to heading of 97 days and plant height of 110 cm which is in tune to CMS line. This similar heading is helpful in synchronised flowering whereas more height in maintainer line facilitates better pollen movement for maximum seed set.

In addition, few additional agro-morphological traits namely coleoptile colour, growth habit, foliage colour, awn colour, spike colour and shape and grain colour were observed over these years which showed similar pattern to the parental maintainer line CBW 38 (Table.2). The results also indicated comparable spikelet number per spike, spike length and maturity period in DCMS 46A as compared to the maintainer line CBW 38. The maturity days was more in the DCMS 46A which is beneficial to get good grain development in the CMS line as well as seed set while attempting hybrid combinations.

DCMS 46A may be used intensively for development of hybrid wheat for Indian conditions so that new breakthrough may be achieved in wheat productivity.

29. DCMS 51A and 51B (IC0638603 & IC0638604; INGR21029), a New CMS (A) line of Wheat (*Triticum aestivum*) in DBW 76 Background with CMS Source (Chuan 18A) along with Maintainer (B) Line.

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Triticum timopheevii based cytoplasm sources were MTSA 2A, Chuan 13A and Chuan 18A which were transferred in the background of diverse CIMMYT advance lines. These CMS lines from CIMMYT were introduced in India as source of cytoplasmic male sterility for hybrid wheat development programme.

The genetic stock DCMS 51A was developed using CHUAN 18A based CMS line CMS21A (CHUAN18A/6/7*KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ) as female parent in first cross with Indian advanced variety DBW 76 as male parent. DBW 76 was a high yielding elite genotype evaluated in NIVT

Table 1: Performance of DCMS 51A and its maintainer for quantitative traits

Year	DCMS51A (CMS- A line)			DCMS 51B (DBW 76: B line)	
	Male sterility (%)	Days to heading	Plant height (cm)	Days to heading	Plant height (cm)
2015-16	100	88	103	92	107
2016-17	100	84	109	84	112
2017-18	100	91	100	88	104
2018-19	100	100	124	95	126
Mean	100	91	109	90	112

Table 2: Performance of DCMS 51A and its maintainer DCMS 51B for agro-morphological traits

Traits	DCMS 51A	DCMS 51B (DBW 76)
Coleoptile Colour	Absent	Absent
Growth habit	Semi erect	Semi erect
Foliage Colour	Green	Green
Spike colour	white	white
Spike shape	Tapering	Tapering
Awn colour	White	White
Grain colour	Amber	Amber
Spike length (cm)	13	13
Spikelet number	23	22
Days to maturity	148	145

1B meant for north western plains zone as well as north eastern plains zone under timely sown irrigated condition. After initial cross, 8 generations of backcrosses were made with DBW 76 as recurrent parent in order to recover its agronomic background. In this process, more than 100 spikes were pollinated. In every generation, five spikes of recipient population were bagged just after emergence from the flag leaf in order to ensure complete male sterility as indicated by no seed set.

After eight backcross generations, the resultant male sterile line was planted in the field along with recurrent parent as maintainer line. The newly developed CMS lines were pollinated with maintainer lines for getting seeds for next generation. For the purpose, the CMS line was planted along with maintainer line in 2B: 4A: 2B row ratio and all the recommended agronomic practices were adopted to raise a good crop during four consecutive crop seasons of 2015-16, 2016-17, 2017-18 and 2018-19. Five randomly selected spikes of these CMS lines were bagged at just after emergence from flag leaf and the seed set was observed in these

un-pollinated spikes for calculating seed set percentage. The data were also recorded on days to heading and plant height for evaluating their suitability to hybrid wheat programme. The seed set was observed among these lines and the results indicated no seed set in the bagged un-pollinated spikes of the CMS lines. This indicated complete male sterility in the new CMS line DCMS 51A (Table 1).

In hybrid development programme based on three-line system, the maintenance of CMS lines is very crucial. The similarity in days to heading and plant height in CMS and maintainer lines is needed to facilitate pollination and get maximum seed set in the CMS line. The heading period in DCMS 51A was ranged from 84-100 days with mean of 91 days whereas average plant height was 109 cm with range of 100-124 cm. Compared to this, the maintainer line DCMS 51B (DBW 76) showed average days to heading of 90 days and plant height of 112 cm which is in tune to CMS line. This similar heading is helpful in synchronised flowering whereas more height in maintainer line facilitates better pollen movement for maximum seed set.

In addition, few additional agro-morphological traits namely coleoptile colour, growth habit, foliage colour, awn colour, spike colour and shape and grain colour were observed over these years which showed similar pattern to the parental maintainer line DBW 76 (Table.2). The results also indicated almost similar spikelet number per spike, spike length and maturity period in DCMS 51A as compared to the maintainer line DBW 76. The maturity days was more in the DCMS 51A which is beneficial to get good grain development in the CMS line as well as seed set while attempting hybrid combinations.

It may be concluded that the genetic stock DCMS 51A may be used intensively for development of hybrid wheat for Indian conditions so that new breakthrough may be achieved in wheat productivity.

30. IC128565 (IC0128565; INGR21030), a Wheat (*Triticum aestivum*) Germplasm Resistant to Leaf Rust.

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A comprehensive germplasm evaluation study of wheat accessions conserved in the Indian National Genebank was conducted to identify sources of leaf rust. Field testing for leaf rust resistance was carried out at 10 different locations Pantnagar (Uttarakhand), Ludhiana (Punjab), Karnal (Haryana), Varanasi (Uttar Pradesh), Kumarganj (Uttar Pradesh), Vijapur (Gujarat), Powarkheda (Madhya Pradesh), Pune (Maharashtra), Dharwad (Karnataka) and Wellington (Tamilnadu) for two years followed by molecular screening to detect the presence of APR genes *Lr34+*, *Lr46+*, *Lr67+* and *Lr68* in Indian wheat germplasm. 190 wheat germplasm lines which were selected from 6319 accessions based on *Ltn* disease screening and average coefficient of infection. Wheat accessions which were found to be resistant in the

field were then assayed for seedling resistance. Molecular analysis of 190 selected germplasm lines was done to identify different combinations of APR genes imparting resistance to leaf rust. 49 accessions were identified which were carrying either two or three APR genes were evaluated for yield stability across four different locations in India viz., Pantnagar, Varanasi, Powarkheda and Pune, using additive main effects and multiplicative interaction (AMMI) model.

Among identified 49 germplasm lines, IC128565 which showed the presence of leaf rust resistance genes *Lr34+* (*Lr34/Yr18/Sr57/Pm38*), *Lr46+* (*Lr46/Yr29/Sr58/Pm39*) and *Lr68* may be considered promising multiple disease resistant germplasm and could be included in breeding program as parents for developing new durable multiple rust resistant cultivars.

31. IC128638 (IC0128638; INGR21031), a Wheat (*Triticum aestivum*) Germplasm Resistant to Leaf Rust and Yield Stability across the Locations

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locations Pantnagar (Uttarakhand), Ludhiana (Punjab), Karnal (Haryana), Varanasi (Uttar Pradesh), Kumarganj (Uttar Pradesh), Vijapur (Gujarat), Powarkheda (Madhya Pradesh), Pune (Maharashtra), Dharwad (Karnataka) and Wellington

(Tamilnadu) for two years followed by molecular screening to detect the presence of APR genes *Lr34+*, *Lr46+*, *Lr67+* and *Lr68* in Indian wheat germplasm. 190 wheat germplasm lines which were selected from 6319 accessions based on *Ltn* disease screening and average coefficient of infection. Wheat accessions which were found to be resistant in the field were then assayed for seedling resistance. Molecular analysis of 190 selected germplasm lines was done to identify different combinations of APR genes imparting resistance to leaf rust. 49 accessions were identified which

were carrying either two or three APR genes were evaluated for yield stability across four different locations in India viz., Pantnagar, Varanasi, Powarkheda and Pune, using additive main effects and multiplicative interaction (AMMI) model.

Among identified 49 germplasm lines, IC128638 which showed the presence of leaf rust resistance genes *Lr46+* (*Lr46/Yr29/Sr58/Pm39*), *Lr67+* (*Lr67/Yr46/Sr55/Pm46*) and *Lr68* may be considered promising multiple disease resistant germplasm and could be included in breeding program as parents for developing new durable multiple rust resistant cultivars.

32. PML 35 (IC0637577; INGR21032), a Maize (*Zea mays*) Germplasm Tolerant to High Density Planting. Stable high yielding (2.29 t/ha) Under normal density. Medium in maturity (95 days).

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Globally, as additional land for maize production is limited, it is essential to increase the maize productivity to cope with the increasing demand for grains. High-density planting is a practical approach for increasing maize yield per unit area (Lee EA, and M Tollenaar, 2007). For instance, the average maize yield is approximately 6,000 kg/ha with a planting density of 52, 500–67,500 plants/ha in China, both parameters are lower than those in the United States. If maize planting density is increased by 15,000 plants/ha, the total maize yield is predicted to be enhanced by 20% (Gong *et al.*, 2015). However, maize varieties/hybrids that are suitable for high-density planting are still lacking in most of the developing countries due to various factors including non availability of suitable genotypes (Andrivon, 2013). Thus, identification of such varieties is an important task in enhancing productivity of maize.

Genotypes were evaluated in randomized block design with two replications. Inter and intra row spacing was adjusted to fit the prescribed density for the evaluation. To attain 66666 plants/ ha, plants were grown in 75 X 20 cm spacing, 60 X 20 cm spacing was followed to attain 83333

plant density per hectare. We ensured the plant density of 88888 plants /ha by following plant geometry of 75 X 15 cm. The highest density of 111111 plants/ha was attained by adjusting genotypes in 60 X 15 cm spacing. Data were recorded on yield component traits.

Among the tested lines, PML 35, PML 46, PML 76, PML 93, KRN-2-5-2, and UMI 1200 significantly out yielded over the trial mean at type III density i.e., 88888 plants/ha. These lines can be designated as density tolerant lines for grain yield. Among the tolerant line for density stress, PML 35 had high yielding ability (4.07 t/ha).

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33. UMI1230B+-1 (IC0637575; INGR21033), a Maize (*Zea mays*) Germplasm with Improved Beta Carotene (9.248µg/g)

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Vitamin A deficiency (VAD) is a global health problem. Many people around the world, especially children and pregnant women are VAD deficient. Maize is known as an important source of provitamin A (Baveja *et al.*, 2020) Hence the enrichment of pro vitamin A in maize through breeding is a best option to alleviate the vitamin A deficiency (Mehta *et al.*, 2020).

For this purpose UMI1230 is an elite maize inbred which is widely adapted to the tropical region and serves as a male parent for CO6 maize hybrid was taken to improve the beta carotene concentration through marker assisted breeding. Donor parent called HP467-15 obtained from CIMMYT, having beta carotene content of 10.525 µg/g was used as a donor parent to transfer the beta carotene trait in to the inbred UMI1230 through marker assisted backcross breeding. Crossing was initiated during *rabi* (2011-12). The BC1F1 and BC2F1 were obtained by backcrossing the F1's and BC1F1's to its recurrent parent (UMI1230) and evaluated in *Kharif* 2012 and *rabi* (2012-2013) respectively, using the gene specific marker crtRB1, to identify the heterozygous plants having both the favourable allele (543bp) and unfavourable allele (296bp). In *Kharif* 2013 the BC2F1 plants were selfed to produce BC2F2. Further BC2F2 plants were raised in *rabi* (2013-14) and were screened for the target allele (543bp) to fix the homozygous plants for beta carotene trait and selfed to produce BC2F3. Background screening was done with 214 polymorphic SSR markers and revealed 90.41% – 92.21% recovery of recurrent parent genome in

BC2F3 and more than 80% of morphological resemblance with its recurrent parent. Similar results in accordance with Chandran *et al.*, 2019. In addition, beta carotene estimation was performed on the parents (UMI1230 and HP467-15) and the identified improved lines using High Performance Liquid Chromatography (HPLC). The mean beta carotene value of recurrent parent from seven locations was 1.468 µg/g and the donor parent was 10.525 µg/g (Senthil *et al.*, 2020). The improved beta carotene line UMI1230β⁺-1 recorded the mean beta carotene content of 9.248 µg/g from seven locations. Thus the improved line recorded mean beta carotene value comparable to that of the donor parent involved in the backcross breeding programme. Also the improved high beta carotene line UMI1230β⁺-1 recorded curved type of leaf attitude, lax spikelet of tassel and flint kernel as in case of recurrent parent (UMI1230).

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34. UMI1200β⁺-2 (IC0637576; INGR21034), a Maize (*Zea mays*) Germplasm with Improved Beta Carotene (8.286 µg/g)

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Maize is an excellent nutritional source and is consumed as a staple food in different parts of the world, including India. Developing maize genotype with high concentration of betacarotene can help to alleviate the problem of vitamin A deficiency (Muthusamy *et al.*, 2014). For this purpose, UMI1200 is an elite maize inbred which is widely adapted to the tropical region and serves as a male parent for CO6 maize hybrid. Donor parent called HP467-15 obtained from CIMMYT, having beta carotene content of 10.525 µg/g was used as a donor parent to transfer the beta carotene trait in to the inbred UMI1200 through marker assisted backcross breeding. Crossing was initiated during *rabi* (2011-12). The BC1F1 and BC2F1 were obtained by backcrossing the F1's and BC1F1's to its recurrent parent (UMI1230) and evaluated in

Kharif 2012 and *rabi* (2012-2013) respectively, using the gene specific marker crtRB1, to identify the heterozygous plants having both the favourable allele (543bp) and unfavourable allele (296bp). In *Kharif* 2013 the BC2F1 plants were selfed to produce BC2F2. Further BC2F2 plants were raised in *rabi* (2013-14) and were screened for the target allele (543bp) to fix the homozygous plants for beta carotene trait and selfed to produce BC2F3. Background screening was done with 214 polymorphic SSR markers and revealed 90.24% – 92.42% recovery of recurrent parent genome in BC2F3 and more than 80% of morphological resemblance with its recurrent parent. Similar results in accordance with Chandran *et al.*, 2019. In addition, beta carotene estimation was performed on the parents (UMI1200 and HP467-15)

and the identified improved lines using High Performance Liquid Chromatography (HPLC). The mean beta carotene value of recurrent parent from seven locations was 0.783 µg/g and the donor parent was 10.525 µg/g (Senthil *et al.*, 2020). The improved beta carotene line UMI1200β⁺-2 recorded the mean beta carotene content of 8.286 µg/g from seven locations. Thus the improved line recorded mean beta carotene value comparable to that of the donor parent involved in the backcross breeding programme. Also the improved high beta carotene line UMI1200β⁺-2 recorded narrow type of leaf attitude, dense spikelet of tassel and dent kernel as in case of recurrent parent used (UMI1200)

35. OIN-456 (IC00503729; INGR21035), a Jute (*Corchorus olitorius*) Germplasm Susceptible to Stem rot disease caused by *Macrophomina phaseolina*

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Among all diseases of jute, stem rot caused by *Macrophomina phaseolina* is economically most serious disease in both cultivated species. Although it's commonly known as stem rot, but any part of the plant may be infected by the pathogen at any stage of growth right from germination to harvest leading to reduced fiber yield and quality. Breeding for host-plant resistance can reduce reliance on chemical fungicides and facilitate environmentally safe organic jute production. Several disease resistant varieties in different crops have been released for commercial cultivation using MAS in different parts of the world.

While breeding for biotic stress resistance, a stable disease resistance and susceptible sources are required to capitalize the molecular markers for identification and mapping of resistant genes/QTLs. Susceptible genetic stocks have several applications in resistance breeding like they were often used as negative control while screening for resistant source, as a source of inoculum for spreading the disease on test accessions in screening plots and for development mapping populations.

In tossa jute (*C. olitorius* L.), resistant sources for stem rot resistance were identified by De and Mandal (2012)

Table 1: Disease reaction of the OIN-456 during 2015-2018 under sick plot conditions expressed as AUDPC values

Germplasm line	Species	2015	2016	2017	2018
OIN-456	<i>C. olitorius</i>	748	1385	609	939
OIN-154-1	<i>C. olitorius</i>	62	201	44	190
WCIN-136-1	<i>C. aestuans</i>	0	0	0	0
WCIJ-150-1	<i>C. fascicularis</i>	0	0	0	0

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and Meena *et al.*, (2015) and in white jute (*C. capsularis* L.) by Mandal *et al.*, (2000). Whereas susceptible source was identified only in capsularis germplasm (Mandal *et al.*, 2000), no stem rot susceptible line was reported in olitorius jute. So, to advance the resistance breeding programmes in tossa jute we have screened around 100 olitorius germplasm lines of different geographic origin through artificial stem inoculation method and selected lines were screened under sick plot conditions. Among the olitorius germplasm OIN-456 is consistently recorded as highly susceptible (Table 1). This line could be useful in developing mapping population for understanding genetics and identification of genes/QTLs responsible for disease resistance. OIN-456 is a red pigmented line with glossy leaf and this line was collected from Madhya Pradesh state (Dandakaranya), India.

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36. WCIN-136-1 (IC0637579; INGR21036), a Jute (*Corchorus aestuans*) Germplasm Highly Resistant to Stem Rot caused by *Macrophomina phaseolina*

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Identification of stable biotic resistance sources has paramount importance in resistance breeding for the development of resistance varieties and identification of resistant genes/QTLs. In jute only few stem rot disease resistance sources were reported. In cultivated tossa jute (*Corchorus olitorius* L.) only moderate stem rot resistance was reported by De and Mandal (2012) and Meena *et al.*, (2015) and resistance in white jute (*Corchorus capsularis* L.) by Mandal *et al.*, (2000). Lack of highly resistant sources and cross incompatibility between *C. olitorius* and *C. capsularis* germplasm lines hampered development of high yielding, stem rot resistant tossa jute varieties.

In this back drop we have identified a wild jute germplasm line (WCIN-136-1) of *Corchorus aestuans* L. with stable disease resistance (zero AUDPC values, Area Under Disease Progressive Curve) reaction during screening for four consecutive years (2015-2018) under sick plot conditions at ICAR-CRIJAF, Barrackpore (Table 1). WCIN-136-1 (*Corchorus aestuans* L.) is a unique wild jute germplasm line with resistant to major disease and insect pest in jute *i.e.* stem rot (caused by *Macrophomina phaseolina* (Tassi) Goid., bihar hairy caterpillar (*Spilosoma obliqua*) resistance and high fiber fineness. WCIN-136-1 was developed through pure line selection from indigenous germplasm line WCIN-136 collected from Sundarban, West Bengal, India.

This germplasm line was first identified as resistant for stem rot disease by artificial stem inoculation method and in subsequent years WCIN-136-1 tested for its stable resistance under sick plot conditions over the years. The genotype

Table 1: Resistant reaction of the WCIN-136-1 during 2015-2018 under sick plot conditions expressed as AUDPC values

Germplasm line	Species	2015	2016	2017	2018
OIN-456	<i>C. olitorius</i>	748	1385	609	939
OIN-154-1	<i>C. olitorius</i>	62	201	44	190
WCIN-136-1	<i>C. aestuans</i>	0	0	0	0
WCIN-150-1	<i>C. fascicularis</i>	0	0	0	0

WCIN-136-1 not only showed resistant to stem rot disease but it also showed resistant reaction to bihar hairy caterpillar with 100% larval mortality and 0% pupation along with high fiber fineness (0.5 tex). So, this line can be useful as a donor parent to transfer both stem rot, bihar hairy caterpillar resistant and fiber fineness to cultivated tossa jute as it is cross compatible with cultivated tossa jute.

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37. SPV 2438 (PSV 316) (IC0637580; INGR21037), a Sorghum (*Sorghum bicolor*) Germplasm High Protein Content (11.73%).

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Sorghum is an important climate resilient crop of dry lands and integral component of varied agro ecologies and cropping systems. In the context of climate change and prevailing protein energy malnutrition there is a need to identify resilient cultivars with enhanced protein content.

Due to systematic breeding efforts at Regional Agricultural Research Station, Palem in collaboration with Indian Institute of Millets Research (IIMR) Hyderabad high yielding culture SPV 2438 was developed. This culture was derived from a cross between SPV-504 x ICSR103 through Pedigree method

of breeding at Regional Agricultural Research Station, Palem, PJTSAU, Telangana state of India. It was evaluated in 15 AICRP trials from 2016 to 2018 and recorded significantly superior grain yield (22.16%) and fodder yield (72.18%) over check CSV 17. The normal range of protein content in white sorghum ranges from 8.5 to 10 percent. Where as in quality analysis SPV2438 recorded highest protein content (11.73%) among all the entries and national checks evaluated during the period at all the locations across the country. This entry also

showed stable and moderately resistant reaction against grain mold with 3.23 field grade and 4.37 threshed grade during three years of AICRP evaluation. SPV 2438 matures, on an average, in 104 days and takes 67 days to flower. It produces semi compact panicles with medium sized grains (seed weight of 2.66 g/100 seeds) of elliptic shape. The culture attains the height of 227 cm with 21.0-28.0 cm length panicles.

38. GMN 16-5 (IC0635028; INGR21038), a Sorghum (*Sorghum bicolor*) Germplasm with Grain Mold Resistance (3.8).

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Sorghum grain molds is an important disease that affects the quality of the produce and ultimately reduces market price making sorghum cultivation non profitable. Heavy rains at the time of maturity cause severe damage to the sorghum grain, which affects its market price. The deterioration is mainly due to infection caused by a complex of fungi, collectively known as "grain molds". The genetics of grain mold resistance is very complex, governed by major and minor genes showing significant GxE interactions and is hampering the progress in the breeding for grain mold resistance. In India, during the past five decades, the area under rainy season sorghum cultivation has come down from 10.9 to 2.7 million hectares, the main reasons for this decline being poor quality of the rainy season produce, low market value and stagnant yields. The problem of grain mold is encountered throughout the humid tropical and subtropical regions. It remains as a major constraint to sorghum production in most parts of India, Africa, Latin America, and USA. It is necessary to develop new varieties or hybrids that possess resistance to this pest.

In order to incorporate resistance into sorghum parental lines, intensive breeding efforts were initiated at IIMR. Since this is a very complex disease involving many traits and high GxE interactions, population breeding was initiated

during 2000 involving elite B and R lines and grain mold resistance sources. Three years of random mating and three cycles of half sib selections were carried out before making selections from the population. Individual selections were made from the population and these were stabilized over 4-5 generations. These stable lines were evaluated for their performance for grain mold resistance and agronomy in station trials.

The seed of the promising selections with grain mold resistance was multiplied and the genotypes were tested in multilocation under AICSIP grain mold nursery.

GMN 16-5 was a population breeding derivative with better level of grain mold tolerance compared to the one of their parents, 296B and agronomically better compared to the grain mold resistant source, B 58586. GMN16-5 was better than the B58586 in desirable agronomic characters like more grain size, less plant height and more compactness of panicles. It is a short and early improved genetic stock with grain mold tolerance better than check, 296B. Bold seed compared to the resistant check, B 58586 with desirable plant height and more compact panicle. GMN 16-5 can be used as resistant source in the grain mold resistance breeding program aiming at development of parental lines and genotypes with grain mold resistance along with agronomic acceptability.

Table 1: Performance of GMN 16-5 for grain mold resistance in AICSIP GMN trials over three years (2016- 3 Env; 2017- 4Env; 2018- 3 Env)

GMN line	Days to flower				Grain mold score (FG)				Grain mold (TG)				100 Seed weight (g)				PC*
	2016	2017	2018	Av	2016	2017	2018	Av	2016	2017	2018	Av	2016	2017	2018	Av	
GMN16-5	68	59	67	65	3.5	3.6	4.2	3.8	3.7	3.0	4.3	3.7	2.1	2.7	2.9	2.6	2.0
B 58586	70	66	72	69	2.2	2.9	1.8	2.3	2	2.3	1.5	1.9	1.8	2.1	1.9	1.9	1.0
Bulk Y	71	63	66	67	6	6.8	7.0	6.6	8	6.3	6.5	6.9	1.7	3.1	3.5	2.8	2.0
296 B	77	-	79	78	5.5	-	5.1	5.3	6.3	-	5.3	5.8	2	-	2.8	2.4	3.0
CD 5%	9.2	5.0	5.8		1.8	0.7	1.3		1.9	1.3	1.8		0.49	0.9	0.6		0.2
CV (%)	6.2	4.3	6.3		22.8	10.9	23.2		29.4	23.9	21.0		11.9	19.5	11.4		4.9

*PC- Panicle compactness

Table 2: Performance of GMN 16-5 for presence of individual grain mold fungi and other traits in AICSIP GMN trials over three years (2016- 3 Env; 2017- 4 Env; 2018- 3 Env)

	Fusarium (%)				Curvularia (%)				Germination (%)				Plant height (cm)		
	2016	2017	2018	Av	2016	2017	2018	Av	2016	2017	2018	Av	2017	2018	Av
GMN16-5	16	16	9	14	26	18	9	17	60	68	80	69	150	130	140
B58586	10	17	4	10	16	21	4	14	80	56	87	74	221	220	221
BulkY	27	14	18	20	23	22	8	18	38	71	71	60	125	100	112
296B	37	22	8	22	16	31	11	19	35	48	72	51	103	103	103
CD 5%	11.5	9.0	6.3		10.1	9.6	4.0		18.0	15.0	10.2		58.7	37.1	
CD 1%	15.5	12.6	8.5		13.6	13.4	5.4		24.2	20.3	13.8		79.6	49.5	
CV (%)	29.5	23.2	44.7		25.1	23.2	23.1		13.7	13.6	6.1		17.7	13.0	

39. SPV 2481 (IC0338975; INGR21039), a Sorghum (*Sorghum bicolor*) Germplasm with High Seed Weight (3.54 g) and High Dry-Fodder Yield (9798 kg/ha)

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Sorghum [*Sorghum bicolor* (L.) Moench] is one of the important dry-land crops of semiarid tropics. It is mainly grown in the drought prone areas to meet the food and fodder security of the region especially Maharashtra, Karnataka, Andhra Pradesh and Telangana where occurrence of drought is very common. Subalakhshmi *et al*, 2019 observed that the characters viz., leaf width, panicle length, and hundred seed weight are important traits for grain yield improvement. Elangovan and Kiran Babu (2015) observed PCV and GCV were higher for 100-seed weight and other traits. High amount of GCV and PCV suggested greater scope for selection of superior genotypes for these traits. Dry fodder yield was positively associated with GFY, plant height, stem girth, the no. of tillers, IVDMD, DDM and protein yield and negatively with panicle length visible above sheath. These traits could be considered as the best selection criteria in sorghum breeding programmes for the development of high yielding varieties (Rohila *et al*, 2020).

The sorghum genotype SPV 2481 was evaluated in the Initial Advanced Varietal Trial (Deep Soil) during Rabi 2016-17 at 14 locations and identified as second genotype for more 100-Seed weight with (3.54 g) as compared to 23 genotypes tested including 4 checks. In addition to that the genotype SPV 2481 was evaluated in Initial Advanced Varietal Trial

(Deep Soil) during Rabi 2016-17 at 14 locations and identified as first genotype for more dry fodder yield with (9798 kg/ha) as compared to 23 genotypes tested including 4 checks.

The sorghum germplasm SPV 2481 has been identified as new sources for seed weight and higher dryfodder yield in post-rainy (*rabi*) sorghum. It has been proved as a source for more seed weight in IAVT during 2016-17. It can be used as a parent in the *rabi* sorghum improvement programme to develop *rabi* varieties.

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40. SPV 2412 (IC0415833; INGR21040), a Sorghum (*Sorghum bicolor*) Germplasm with High Seed Weight (3.61 g) and High Dry-Fodder Yield (9720 kg/ha)

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Sorghum [*Sorghum bicolor* (L.) Moench] is one of the important dry-land crops of semiarid tropics. It is mainly grown in the drought prone areas to meet the food and fodder security of the region especially Maharashtra, Karnataka, Andhra Pradesh and Telangana where occurrence of drought is very common. Subalakhshmi *et al*, 2019 observed that the characters viz., leaf width, panicle length, and hundred seed weight are important traits for grain yield improvement. Elangovan and Kiran Babu (2015) observed PCV and GCV were higher for 100-seed weight and other traits. High amount of GCV and PCV suggested greater scope for selection of superior genotypes for these traits.

The sorghum genotype SPV 2412 was evaluated in the Initial Advanced Varietal and Hybrid Trial (Deep Soil) during Rabi 2015-16 at 10 locations and identified with third genotype for more 100-Seed weight with (3.00 g) as compared to 28 genotypes tested including 5 checks (IIMR Publication Number 1/2016-17). In the next year SPV 2412 was evaluated in Initial Advanced Varietal and Hybrid Trial (Deep Soil) during Rabi 2016-17 at 14 locations and identified with first genotype for more 100-Seed weight with (3.61 g)

and superior fodder yield (6593 kg/ha) as compared to 23 genotypes tested including 4 checks (IIMR Publication Number 1/2017-18).

The sorghum genotype SPV 2412 is a selection from the local landrace E 142 (IC 415833) from Uttar Pradesh has consistently proved as a potential sorghum genotype for more seed weight in IVT and AVT trials. It can be used as a parent in the sorghum improvement programme.

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41. SPV 2612 (IIMR Red); E-142 (IC0415833; INGR21041), a Red Grain ed Sorghum (*Sorghum bicolor*) Germplasm with High Tannin Content Of (4.51mg ce/g). Adaptability to both *Kharif* and *Rabi*. High Grain Yield

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SPV 2612 is an outcome of the colored sorghum improvement program at Indian Institute of Millets Research, Hyderabad aiming at developing high yielding red sorghum varieties. To promote colored sorghum improvement for feed industry and export purpose, breeding was initiated to develop high yielding colored sorghum lines. The project developed stable lines in different grain color background. To develop red sorghum lines, a number of germplasm lines with red grain were selected from the gene bank and they were crossed to the elite lines. The line SPV 2612 was derived from one such cross between a popular grain sorghum variety, CSV 15 which has white grain and the red germplasm line IS 23514 from Sudan belonging to the race, *Caudatum*. The

initial crossing program was made during *rabi* 14 and the F1, F2 and further generations were raised subsequently. In each generation, selections were made based on the color of the grain, maturity duration and productivity.

The selected lines were stabilized and a station trial of the selected lines along with checks was taken up during *kharif* 2017. In the station trials, IIMR red, which was the name given to SPV 2612 at station, was evaluated in a trial where twenty-five colored sorghum genotypes were evaluated in RCBD with CSV 20 as check. Preliminary evaluation identified red sorghum lines with grain yield on par with the high yielding white grain sorghum. IIMR red, a derivative of CSV 15 crossed with a colored germplasm line, IS 23514 was the

Table 1: Performance of SPV 2612 (IIMR Red) in AICSIP trials over two years (2018-Kharif 4 env, Rabi- 5 env; 2019-Kharif 4 env, Rabi 5env)

Genotype	Grain Yield (kg/ha)						100 Seed weight (g)				Av
	Kharif			Rabi			Kharif		Rabi		
	2018	2019	Av	2018	2019	AV	2018	2019	2018	2019	
SPV 2612	4761.9	4426.2	4594	3103	2837	2970	2.99	2.51	2.7	2.64	2.71
CSV 20	4597.1	3262.8	3929.9	2550	2864	2707	3.01	2.42	2.42	1.96	2.45
CD 5%	1188	1417.1		700	503		0.38	0.43	0.48	0.29	
CV (%)	16.67	15.0		22.57	10.4		9.97	13.15	15.03	10.9	

Table 2: Performance of SPV 2612 (IIMR Red) in AICSIP trials for biochemical traits in 4 environments (2018-2 env; 2019-2 env)

Genotype	Protein			Tannin		
	2018	2019	Av	2018	2019	Av
SPV2612	9.88	8.2	9.04	4.48	4.53	4.51
CSV 20	8.99	7.14	8.07	0.80	1.44	1.12
CV	7.54	9.21		105.5	54.9	
CD	2.38	1.96		7.14	2.93	

best line with yield numerically and better than CSV 20 and bold seed. Then this entry was submitted for multilocation evaluation in AICRP- Sorghum which was tested under the

specialty sorghum category. It was tested in a total of 18 environments over two years. It was tested in 4 locations during *kharif* 2018; 5 locations in *rabi* 2015; 4 locations in *kharif* 2019 and 5 locations in *rabi* 2019. In both the seasons, SPV 2612 has given grain yield on par with the popular variety, CSV 20 (Table 1).

SPV 2612 was analyzed for biochemical constituents like protein and tannin for samples from 4 environments: two in 2018 and two in 2019 and it was found to have high tannin content (Table 2). Tannin content indicates the presence of polyphenols and antioxidant activity which is very good for human health

42. EJN 11 (IC0585181) (IC0585181; INGR21042), a Sorghum (*Sorghum bicolor*) Germplasm with Early Flowering (<56 days).

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Sorghum [*Sorghum bicolor* (L.) Moench] is one of the important dry-land crops of semiarid tropics. It is mainly grown in the drought prone areas to meet the food and fodder security of the region especially Maharashtra, Karnataka, Andhra Pradesh and Telangana. Ezeaku, *et al.*, (1999) evaluated 352 accessions of sorghum germplasm originated from Nigeria and Chad and classified into three flowering groups viz., early medium and late flowering. Elangovan, *et al.*, (2013) reported that preliminary characterization of 107 accessions of sorghum germplasm during 2008-2010 and identified EJN 11 one of the early maturing germplasm.

The sorghum germplasm EJN 11 (IC0585181) was evaluated at the AICRP on Sorghum- Deesa centre during *Kharif* 2010 and identified as early flowering germplasm as compared to 103 *kharif* landraces along with 3 checks. The germplasm EJN 11 (IC0585181) again evaluated in AICRP on Sorghum-Deesa, Indore and Hyderabad during *Kharif* 20110 and identified as early flowering germplasm across three

locations as compared to 103 *kharif* landraces along with 3 checks. The local name of EJN 11 (IC0585181) is *Utavali* and collected from Kankraj taluk, Banaskantha district of Gujarat during 2009. This is mainly used for fodder purpose.

The sorghum germplasm EJN 11 (IC0585181) has been identified as new sources for early flowering and can be used in the sorghum improvement programme to develop early variety.

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43. AKGMR 117 (IC0637581; INGR21043), a *kharif* Sorghum (*Sorghum bicolor*) Germplasm with Grain Mold Resistance with Field Grade Grain Mold Score of 3.10 and Threshed Grade Grain Mold Score of 3.53.

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Sorghum (*Sorghum bicolor*) is the fourth most important cereal following rice, wheat and maize and staple food in the semi-arid parts of the world. Grain mold is the major biotic constrain in the way of production, marketing and utilization of *kharif* grain sorghum. It is one of the most important diseases of sorghum in many countries in Asia, Africa, North America, South America. The term Grain Mold Disease Complex (GDMC) has been used in few instances to describe this disease conditions (Prom *et al.* 2003). The disease is particularly important on improved, short- and medium-duration sorghum cultivars that mature during the rainy season in humid, tropical and subtropical climates. Several fungal species of the genera *Fusarium*, *Curvularia*, *Alternaria*, *Phoma*, *Bipolaris* and *Colletotrichum* have been reported to be associated with grain mold. *Curvularia lunata* and *Fusarium moniliforme* secrete amylase, cellulose and pectinases resulting in the disintegration of endosperm and germ tissues. These fungi also interfere with carbohydrate translocation to developing kernels causing reduction in size and weight of the kernels and ultimately germination of these grains. Therefore, identification of the resistant sources of grain mold in *kharif* sorghum is of prime importance. Considering this, a multilocation and multi season grain mold screening trial entitled "National Grain Mold Nursery (NGN)" was formulated at national level by Indian Institute of Millets Research (IIMR), Hyderabad.

A national level trial was formulated by Indian Institute of Millets Research (IIMR), Hyderabad consisting of the entries contributed by the all the major centers of the AICSIP (All India Coordinated Sorghum Improvement Project) and conducted during the three years of 2016, 2017 and 2018 at the hot spot centers for grain mold i.e. Akola, Parbhani and Dharwad. In this connection, massive breeding programme for grain mold resistance has been continued at AICSIP Akola center and by utilizing the resistance sources identified in the

programme, grain mold resistant lines have been developed at Akola center. AKGMR 117 contributed by Sorghum Research Unit, Dr. PDKV, Akola was tested for three years in the "National Grain Mold Nursery (NGN)" trial formulated at national level by Indian Institute of Millets Research (IIMR), Hyderabad. Trial consisted of the testing entries along with one resistant check for grain mold (B 58586) in loose panicle background and one susceptible check (Bulky yellow). The experiments were sown in randomized block design with three replications.

For the character grain mold field grade the resistant check B 58586 recorded the lowest grain mold infestation of 2.29, while the susceptible check exhibited the highest grain mold field grade of 6.66. The best promising entry was AKGMR117 with the grain mold field grade of 3.10 followed by AKGMR 118 (3.50) and AKGMR 119 (3.65). For the character grain mold threshed grade, the resistant check B 58586 recorded the lowest grain mold infestation of 1.83, while the susceptible check exhibited the highest grain mold field grade of 6.83. The best promising entry was AKGMR117 with the grain mold threshed grade of 3.53 followed by AKGMR 118 (3.45) and AKGMR 119 (4.08).

The promising genotypes AKGMR 117 and the resistant check B 58586 exhibited Grain mold disease resistant reaction towards both field grade mold rating and threshed grain mold rating. This resistant reaction can be attributed to the lower percentage of both the fungi i.e. *Fusarium* and *Curvularia*. Thus, it was concluded from the present study that considering the resistant reaction as confirmed by AICSIP three years report against the grain mold disease, the promising genotype AKGMR 117 showing Grain mold disease resistance need to be exploited in breeding for grain mold resistance in sorghum as the donor parent in the crossing programme.

44. VR 1062 (IC0635026; INGR21044), a Finger Millet (*Eleusine coracana*) Germplasm with Neck Blast Resistance (2.5%) and Finger Blast Resistance (3.0%).

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Finger millet, VR 1062 is neck and finger blast resistant line developed by crossing blast resistant germplasm GE 3076 with improved breeding line VR 855. The cross was performed in 2004 for developing blast resistant high yielding variety and subsequent selections were made through pedigree method from F₂ to F₆. At F₇ stage, it was promoted to Preliminary Yield Trial in 2011. It comes to maturity earlier than check Sri Chaitanya (VR 847) and is on par with Champavathi (VR 708). The plant height is short

with medium number of productive tillers/plant. It has more number of fingers/ear head compared to both the checks.

The entry, VR 1062 along with other test entries and two checks were tested under high disease pressure under field conditions for consecutive five years from 2014 to 2018. It ranked first among all the 3000 entries tested for neck blast resistance (2.5% Pooled data) and also showed resistance to finger blast (3.0% Pooled data). VR 1062 has recorded -37.5% &

-97.41 % (less incidence) of neck blast over resistant check, Sri Chaitanya (VR 847) & susceptible check, Champavathi (VR 708) respectively while it recorded 23.08% & 96.85% less incidence of finger blast over Sri Chaitanya (VR 847) &

Champavathi (VR 708) respectively. Hence, VR 1062 is unique in terms of neck and finger blast resistance and moreover it is a breeding line and hence it can be used directly for neck and finger blast resistance breeding.

45. FMV1155 (IC00403065; INGR21045), a Finger Millet (*Eleusine coracana*) Germplasm with Early Flowering (65 days), Early Maturity (105 days) and Higher Grain Yield (2188 kg/ha)

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Finger millet has the highest grain productivity among millets and the crop can thrive under a variety of harsh environmental conditions. The crop is also known for its nutritional superiority and the notable is highest calcium content (344 mg/100g) of grains which is about 10-15 times than that of any cereal. Breeding short duration (95-100 days) varieties with desirable grain and fodder yield is one of the major objectives in finger millet. Short duration varieties with comparable yield can fit multiple cropping systems and limited water resources and other inputs

Morpho-agronomic characters: The line FMV1155 was tested in All India Coordinated Small Millets Trial during 2018. The mean days to 50% flowering recorded by the entry is 65 days as compared to the similar early maturing check VL376 which recorded 69 days. For days to maturity, the mean days to maturity recorded by the entry is 105 days as compared to the early maturing check VL 376 which recorded 112 days. The entry also recorded higher grain yield of 2188 kg/ha as against the similar maturity check VL 376 which recorded 2155 kg/ha

Associated characters: The genotype FMV1155 recorded mean fodder yield of 8261 kg/ha as compared to similar maturing check VL376 which recorded fodder yield of 8075 kg/ha. The entry recorded plant height of 96 cm (semi-dwarf). The mean number of productive tillers recorded by FMV 1155 is three which is same as the check VL376. The entry also recorded highest ear finger length (9 cm) as compared to check VL 376 which recorded 8 cm. The mean number of ears recorded by FMV1155 is 6 which is similar to check VL376. The line can be an important genetic stock while breeding for early duration variety with high grain yield especially suitable for growing in the Northern finger millet growing regions of the country.

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46. VL 384 (IC0637583; INGR21046), a Finger Millet (*Eleusine coracana*) Germplasm with White Grain. Blast Resistance, Medium Maturity and High Grain Yield

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Finger millet is an annual self-pollinated crop grown in Asia and Africa. In India, finger millet is grown in 1.2 million ha area with average productivity of 1706 kg/ha (Joshi *et al.* 2021). Most of the finger millet production is from brown grain varieties except few areas like Karnataka, Maharashtra, Jharkhand and Orissa where state released white grain varieties are cultivated in small scale. All the available white grained finger millet genotypes are late maturing and highly

blast susceptible. Blast caused by *Magnaporthe grisea* is a major constraint to finger millet production (Sood *et al.*, 2019). Blast affects finger millet at all stages of growth and most of the landraces and a number of varieties are highly susceptible. Brown grained resistant genotypes have been developed and released for cultivation. However, the dark colour of grains has been the major hindrance for its acceptability in baking and food industry (Sharathbabu *et*

Table 1: Distinguishing characteristics of VL 384 compared to checks in All India Coordinated trials from 2013-2015

Character name	VL 384 (White grain)	VL Mandua 352 (Brown grain)	VR 708 (Brown grain)	GPU 45 (Brown grain)
Pigmentation on node	Absent	Absent	Absent	Absent
Growth habit	Erect	Erect	Erect	Erect
Leaf blade pubescence	Present	Present	Present	Present
Number of fingers/ear	7	8	7	7
Ear length (cm)	7.2-7.7	7.3-7.7	6.3-6.4	6.6-7.2
Days to Maturity	110-116	103-107	101-106	104-112
Plant height (cm)	93-97	94-98	84-90	88-96
Grain color	White	Copper Brown	Copper Brown	Copper Brown
Reaction to finger blast (%) (mean of MLT conducted from <i>kharif</i> 2013-2015)	9.15	8.10	15.38	5.35
Reaction to neck blast (%) (mean of MLT conducted from <i>kharif</i> 2013-2015)	9.66	9.45	18.38	5.23

al., 2008). Thus, the preference for white grain finger millet genotypes is increasing recently in the urban areas and baking industry because of low tannins, high protein, low fibre, and higher consumer acceptability (Sharathbabu *et al.*, 2008). However, the available white grain types have been observed to be late maturing and highly susceptible to neck and finger blast in comparison to brown grain types. Till date there is no white grain finger millet released genotype available in the early and medium group in the country matching to brown grain for resistance as well as yield potential.

VL 384 has been indigenously developed from a cross between OUAT 2 (White grain late maturing variety released for the State of Orissa, blast susceptible) and GE 4415 (Early maturing core germplasm line from Bihar). It has maturity duration of 110-116 days and found resistant to finger (9.15%) blast disease compared to the early maturing (101-106 days) brown grain national check VR 708 (15.38%) in all India coordinated trials (*kharif* 2013-2015). Likewise, the percent damage due to neck blast in VL 384 (9.66%) was very low

compared to the early maturing brown grain check VR 708 (18.38%). VL 384 fell in the same disease scale category of 5-10% with other brown grain national check varieties (VL 352 and GPU 45) in all India coordinated trials. Moreover, its average grain yield over the years and locations in coordinated trials was 27.19 q/ha which was 5.10 per cent higher than the best brown grained national check VL *Mandua* 352 (25.87 q/ha).

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47. IE-2871 (IC0473958; INGR21047), a Finger Millet (*Eleusine coracana*) Germplasm Resistant to Neck Blast (3.71 score in 1-9 scale)

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Blast of finger millet, *Pyricularia grisea*, causes significant loss of production worldwide. The average loss has been reported to be around 28–36% and as high as 80–90% in endemic areas (Ramappa *et al.*, 2002; Nagaraja and Mantur, 2007). So far there was no stable source of resistance available against finger and neck blast. 'IE-2871 selection' was a pure line selection from the Zambian germplasm IE-

2871. Selections were made under disease pressure (finger and neck blast) situations at Hyderabad. This selection along with 114 other finger millet genotypes including germplasm of African and Asian origin and a few improved cultivars were evaluated for finger and neck blast resistance across three locations in India representing major finger millet areas. Evaluation was conducted under natural field conditions,

Table 1: Performance and stability of resistance of IE-2871 for finger and neck blast resistance over four environments (Source: Das *et al.* 2021: Crop Protection, 139)

Genotype	Finger blast (1-9 scale)							Neck Blast (1-9 scale)						
	H'16	H'17	V'17	B'18	Mean	<i>b</i> †	<i>S</i> ² di†	H'16	H'17	V'17	B'18	Mean	<i>b</i> †	<i>S</i> ² di†
IE-2871	3.5	2.5	4.0	2.5	3.1	0.80	-0.04	2.0	2.0	3.0	2.0	2.3	0.90	-0.16
IE-2883	2.5	2.5	3.5	3.0	2.9	0.91	-0.03	2.0	2.0	4.0	2.0	2.5	1.08	-0.11
GPU-67	4.6	5.0	4.5	2.0	4.0	1.87	-0.19	3.5	3.5	5.5	2.0	3.6	0.83	-0.14
GE-4449 (RC)	3.5	3.5	4.0	3.5	3.6	0.11	-0.29	3.0	3.5	3.5	3.0	3.3	-0.45	-0.17
UM (SC)	8.5	8.0	7.0	6.0	7.4	0.89	0.24	6.5	6.5	6.5	5.5	6.3	0.32	0.16
CD (5%)	0.96	0.93	0.48	0.55				0.81	0.51	0.43	0.48			

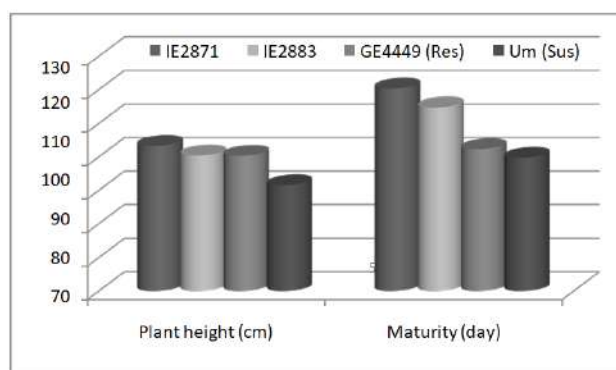
Disease severity was measured on a 1–9 scale, where 1= <1% plants/finger (plant for NB; finger for FB) infected with disease, and 9= >75% infected. H'16: Hyderabad 2016, H'17: Hyderabad 2017, V'17: Vizianagaram 2017, B'18: Bengaluru 2018. †*b*: regression coefficient; †*S*²di: variance deviation regression.

supplemented with artificial inoculation of earhead with blast pathogen at pre-anthesis stage. IE-2871 was stable for both finger and neck blast resistance. Its resistance to neck blast is far superior (2.3 on a scale of 1-9) than the best available resistance source GE-4449 (3.3) (Table 1) (Das *et al.*, 2021). Additionally it also possesses better resistance for finger blast (3.1) than the resistant check GE-4449 (3.6).

IE-2871 also possesses desirable agronomic characters like medium plant height (103 cm), medium maturity (120 days) compared to early maturity in the presently available resistant check GE-9994 (99 days). Medium maturity is a desirable trait in finger millet. This stable sources of neck blast resistance in finger millet, will serve as an important genetic material in a finger millet resistance breeding programme.

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Das IK, KB Palanna, TSSK Patro, KN Ganapathy, Sunil Kumar, N Kannababu and VA Tonapi (2020) Multilocal evaluation of blast resistance in a diverse panel of finger millet in India. *Crop Prot.* **139**: <https://doi.org/10.1016/j.cropro.2020.105401>.

**Figure 1:** Agro-morphological characteristics of IE-2871

Nagaraja A, and AG Mantur (2007) Screening of *Eleusine coracana* germplasm for blast resistance. *J. Mycopathol. Res.* **45**(1): 66–68.

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48. IE-2883 (IC00473970; INGR21048), a Finger Millet (*Eleusine coracana*) Germplasm Resistant to Finger Blast (2.9 score in 1-9 scale)

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Blast of finger millet, *Pyricularia grisea*, causes significant loss of production worldwide. The average loss has been reported to be around 28–36% and as high as 80–90% in endemic areas (Ramappa *et al.*, 2002; Nagaraja and Mantur, 2007). So far there was no stable source of resistance available against finger and neck blast. 'IE-2883 selection' was a pure line selection from the Zambian germplasm IE- 2883. Selections were made under disease pressure

(finger and neck blast) situations at Hyderabad. This selection along with 114 other finger millet genotypes including germplasm of African and Asian origin and a few improved cultivars were evaluated for finger and neck blast resistance across three locations in India representing major finger millet areas. Evaluation was conducted under natural field conditions, supplemented with artificial inoculation of earhead with blast pathogen at pre-anthesis stage. IE-2883

Table 1: Performance and stability of resistance of IE-2871 for finger and neck blast resistance over four environments (Source: Das *et al.* 2021: Crop Protection, 139)

Genotype	Finger blast (1-9 scale)							Neck Blast (1-9 scale)						
	H'16	H'17	V'17	B'18	Mean	<i>b</i> _i [†]	<i>S</i> ² <i>d</i> _i [†]	H'16	H'17	V'17	B'18	Mean	<i>b</i> _i [†]	<i>S</i> ² <i>d</i> _i [†]
IE-2871	3.5	2.5	4.0	2.5	3.1	0.80	-0.04	2.0	2.0	3.0	2.0	2.3	0.90	-0.16
IE-2883	2.5	2.5	3.5	3.0	2.9	0.91	-0.03	2.0	2.0	4.0	2.0	2.5	1.08	-0.11
GPU-67	4.6	5.0	4.5	2.0	4.0	1.87	-0.19	3.5	3.5	5.5	2.0	3.6	0.83	-0.14
GE-4449 (RC)	3.5	3.5	4.0	3.5	3.6	0.11	-0.29	3.0	3.5	3.5	3.0	3.3	-0.45	-0.17
UM (SC)	8.5	8.0	7.0	6.0	7.4	0.89	0.24	6.5	6.5	6.5	5.5	6.3	0.32	0.16
CD (5%)	0.96	0.93	0.48	0.55				0.81	0.51	0.43	0.48			

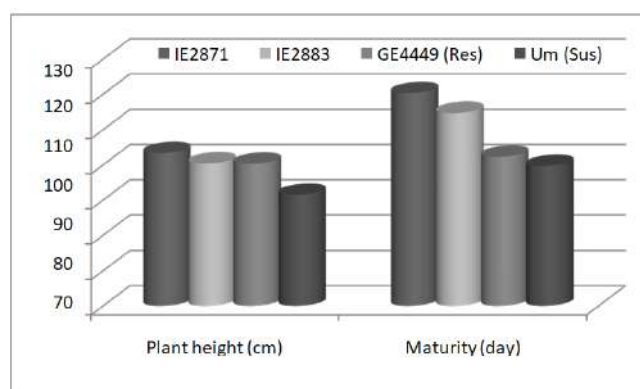
Disease severity was measured on a 1–9 scale, where 1 = <1% plants/finger (plant for NB; finger for FB) infected with disease, and 9 = >75% infected. H'16: Hyderabad 2016, H'17: Hyderabad 2017, V'17: Vizianagaram 2017, B'18: Bengaluru 2018. [†]*b*_i: regression coefficient; [†]*S*²*d*_i: variance deviation regression.

was stable for both finger and neck blast resistance. Its resistance to finger blast is far superior (2.9 on a scale of 1-9) than the best available resistance source GE-4449 (3.6) (Table 1) (Das *et al.*, 2021). Additionally, it also possesses better resistance for neck blast (2.5) than the resistant check GE-4449 (3.6).

IE-2883 also possesses desirable agronomic characters like semi-compact earhead, medium plant height (100 cm), medium maturity (115 days) compared to early maturity in the presently available resistant check GE-9994 (99 days). Medium maturity is a desirable trait in finger millet. This stable sources of neck blast resistance in finger millet, will serve as an important genetic material in a finger millet resistance breeding programme.

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Das IK, KB Palanna, TSSK Patro, KN Ganapathy, Sunil Kumar, N Kannababu and VA Tonapi (2020) Multilocal evaluation of blast resistance in a diverse panel of finger millet in India. *Crop Prot.* **139**: <https://doi.org/10.1016/j.cropro.2020.105401>.
Nagaraja A, and AG Mantur (2007) Screening of *Eleusine coracana*

**Figure 1:** Agro-morphological characteristics of IE-2883

germplasm for blast resistance. *J. Mycopathol. Res.* **45**(1): 66–68.

Ramappa HK, CR Ravishankar, P Prakash (2002) Estimation of yield loss and management of blast in finger millet (ragi). In: Proceedings of Asian Congress of Mycology and Plant Pathology, 1–4 October 2002, University of Mysore, Mysore, 195 p.

49. VB 19-16 (IC0637584; INGR21049), a Barnyard Millet (*Echinochloa esculenta*) Germplasm with Awnless Panicle in the Genetic Background of Japanese Barnyard Millet Species (*E. esculenta*). Semidwarf. Green Glumes

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Towards the development of new plat types, isolation of awnless semi-dwarf genotype of Japanese barnyard millet (*E. esculenta*) is the promising development at ICAR-Vivekananda Institute of Hill Agriculture, Almora, India. In Indian barnyard millet (*E. frumentacea*), lodging is a major production constraint causing substantial losses in grain yield. In general, Japanese barnyard millet (*E. esculenta*)

genotype PRJ 1 (originally ICRISAT gen bank accession, IEC 542) exhibit semi-dwarf growth habit (tolerant to lodging), posses' panicles with large awns and well adapted to North West Himalaya (Sood *et al.*, 2015). However, introgression of these traits in Indian barnyard millet (*E. frumentacea*) (cultivar VL 207) through interspecific hybridization was largely failed due to the presence of strong incompatibility

barriers between the species (Sood *et al.*, 2020). Therefore, intraspecific hybridization between the diverse genotypes of *E. esculenta* is one potential strategy to develop transgressive segregants for agronomic traits. Keeping this in view, both way crosses were attempted between two genotypes (PRB 903 and PRJ 1) of *E. esculenta* at ICAR- VPKAS, Almora to exploit their high yield potential and agronomic superiority. The effort has resulted in obtaining F₆ progenies, characterized by large awnless panicles (27.5–35cm), more number of panicle branches (> 30) and medium plant height (120–148 cm) compared to the parents. In general, panicles of *E. esculenta* genotypes are characterized by large awns. Interestingly, the intraspecific hybridization attempt resulted in development of stable awnless segregants in the genetic background of PRJ 1, which are more vigorous

than parental line. To the best of our knowledge, this is the first report of isolating awnless Japanese barnyard millet genotypes through hybridization. These awnless *E. esculenta*/*E. esculenta* derivatives with reduced plant height with uncompromised fodder potential are the positive developments towards generating dual purpose genetic material of barnyard millet.

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50. IIMR FxM-5 (FXV 632) (IC0479823; INGR21050), a Foxtail Millet (*Setaria italica*) Germplasm with Early Flowering (44 days). Early Duration (76 days) with Desirable Grain Yield.

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Foxtail millet (*Setaria italica* (L.) Beauv.), a self-pollinating cereal, belonging to Family *Poaceae* is one of the oldest of the cultivated millets in the world. It is cultivated in about 23 countries in Asia, Africa and America. It is adapted to a wide range of elevations, soils and temperatures. At present globally it is distributed in Eurasia, Ethiopia, Zimbabwe, Japan, China, Nepal and India with around 4 - 5 m ha area. Foxtail millet is grown for food, feed and fodder purpose. Because of the drought tolerance, it was once an indispensable crop of vast rainfed areas in semi-arid regions in India. The area under this crop in India has come down by more than half during 1990's mainly due to introduction of more remunerative crops like sunflower and soybean in black soils. At present, foxtail millet is cultivated on a limited area in Andhra Pradesh, Telangana, Karnataka, Maharashtra, Tamil Nadu, Rajasthan, Madhya Pradesh, Uttar Pradesh and North Eastern states. It is mostly grown to meet the domestic needs of the rural people. It is widely used as an energy source for pregnant and lactating women, and also for sick people and children, and especially for diabetics. Foxtail millet is highly nutritious and even superior to rice and wheat in certain constituents. Grains of foxtail millet have low glycaemic index (GI) and high fibre (8%) content, because of which it is gaining popularity as diabetic food. The protein content is higher (12.3%) among millets. Because of short duration foxtail millet is ideal as a contingency crop whenever the monsoon delays in semi-arid regions. A number of high yielding varieties have been released, but

the yield potential is not fully expressed in farmers' fields due to various management aspects.

There is a need to develop further high yielding early maturing varieties through recombination breeding, which can mature early enough to avoid late season moisture stress in regions where monsoon withdraws early. Such varieties can utilize the short duration moisture availability, especially when onset of monsoon is delayed, and still can provide grains and fodder to the dryland farmers.

Morpho-agronomic characteristics: IIMR FxM-5 (FXV 632) is a foxtail millet selection from GS 957 with significantly early flowering (44 days) and early maturity (76 days), with desirable productivity (2664 kg/ha). Out of 14 locations tested, in 10 locations it flowered early compared to all other entries and in 6 locations it matured first as well as at all India level (Table 1 a & b). The average per day productivity in this genotype was 35.05 kg/ha/day compared to check, DHFt 109-3 (34.38 kg/ha/day) (Table 2). It has cylindrical panicles with medium compact inflorescence lobes and good yield potential. It can be used as a donor parent for incorporating earliness in recombination breeding to combine high yield and short duration. The early flowering and maturity can help in avoiding terminal moisture stress in locations where rainy season withdraws early. Also in regions where foxtail millet followed by pulses cropping pattern is followed, this helps in early sowing of second crop thus ensuring better germination and crop stand.

Associated characters: The shoot fly dead hearts in IIMR FxM-5 (FXV 632) at 21 DASE was 20.52% compared to 23.68% in resistant check. It recorded 3% less incidence of dead hearts compared to SiA 3179 (23.68%), resistant check and 5% lesser than the susceptible check (SiA 907). The tolerance

level was on par with the national checks. IIMR FxM-5 (FXV 632) will be highly beneficial as a donor parent for breaking the yield ceiling and further foxtail millet improvement and consolidation of gains already achieved through conventional breeding.

Table 1a: Flowering duration of IIMR FxM-5 (FXV 632) Vs. checks

Trait	Location	Variety IIMR FxM-5 (FXV 632)	Rank	Check variety 1 (SiA 3156)	Rank	Check variety 2 (DHFt 109-3)	Rank	CD (5%)
Days to flowering	Nandyal	45	2	48	4	51	15	3
	Vizianagaram	40	1	48	15	46	11	2
	Andhra Pradesh	43	1	48	10	49	13	3
	Dholi	41	1	46	8	49	11	7
	Ranchi	43	2	415	6	50	16	2
	Bangaluru	50	1	52	4	55	13	3
	Hagari	42	1	58	20	49	9	-
	Hanumanamatti	43	1	51	15	49	11	2
	Mandya	38	1	46	22	46	20	5
	Karnataka	43	1	50	14	50	16	3
	Dindori	42	1	49	20	47	8	2
	Rewa	47	3	50	7	51	16	4
	Madhya Pradesh	45	1	50	20	49	11	3
	Solapur	44	1	50	9	52	14	3
	Athiyandal	51	20	49	16	49	14	7
	Hyderabad	51	1	53	3	60	16	5
	Palem	42	1	46	2	53	18	6
	Telangana	47	1	50	2	57	19	5
	All India	44	1	49	9	51	16	2

Table 1b: Maturity duration of IIMR FxM-5 (FXV 632) Vs. checks

Trait	Location	Variety IIMR FxM-5 (FXV 632)	Rank	Check variety 1 (SiA 3156)	Rank	Check variety 2 (DHFt 109-3)	Rank	CD (5%)
Days to Maturity	Nandyal	75	2	78	4	81	16	3
	Vizianagaram	69	1	78	15	77	10	3
	Andhra Pradesh	72	1	78	10	79	12	3
	Dholi	74	1	78	4	81	13	6
	Ranchi	75	1	80	15	80	12	3
	Bangaluru	88	1	91	4	94	13	3
	Mandya	76	11	76	13	76	13	6
	Karnataka	82	5	84	8	85	14	5
	Dindori	75	2	81	20	78	10	3
	Rewa	74	3	76	5	76	7	4
	Madhya Pradesh	74	1	78	18	77	8	4
	Solapur	72	1	86	5	90	20	5
	Athiyandal	84	21	79	3	83	17	5
	Palem	75	1	78	3	84	15	6
	All India	76	1	80	6	82	13	2

Table 2: Grain yield and per day productivity in IIMR FxM-5 (FXV 632) vs. Checks

No.	Entry	Centre code	Grain yield (kg/ha)	Days to maturity	Rank	Per day productivity (kg/ha/day)
1	FXV 606	SIA 3220	2700	81	12	33.22
2	FXV 607	FXV 607	2744	81	8	34.03
3	FXV 615	SIA 3159	2823	82	14	34.62
4	FXV 616	IIMR FxM-2	2718	81	9	33.59
5	FXV 620	GPUF 2	2727	81	15	33.63
6	FXV 622	FXV662	2666	80	7	33.19
7	FXV 624	PKS 22	2577	80	4	32.03
8	FXV 625	SIA 3303	3104	79	2	39.54
9	FXV 626	SIA 4200	2925	83	22	35.11
10	FXV 627	GPUF 3	2753	82	16	33.39
11	FXV 628	GPUF 4	2997	83	18	36.23
13	FXV 630	TNSI 364	2682	81	11	33.31
14	FXV 631	IIMR FxM-4	2785	83	21	33.62
15	FXV 632	IIMR FxM-5	2664	76	1	35.05
16	FXV 633	IIMR FT 1	2537	79	3	32.08
17	FXV 634	FOXTAIL	2422	83	20	29.10
18	FXV 635	DHFT 109-3-1	2854	82	19	34.60
19	FXV 636	DHFT 109-3-2	2987	82	17	36.39
20	DHFt 109-3 (C)		2817	82	13	34.38
21	SiA 3156 (C)		2938	80	6	36.68
	Mean		2789	81		34.43
	CD (5%)		349	2		-

51. IPU19-27 (IC0636672; INGR21051), a Black gram (*Vigna mungo*) Germplasm, Extra Early (60-62 days) Resistant to Yellow Mosaic Disease (MYMV)

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Urdbean [*Vigna mungo*] also known as blackgram, is an important pulse crop among *Vigna* pulses including mungbean and cowpea in India. Under varying climate change scenario short duration pulses are in demand in different growing regions. Under the All India Co-ordinated Research Project (AICRP) several varieties have been released which are meeting the national and local needs. Initially, semi-determinate to indeterminate plant types alongwith longer duration (90-120 days) were desirable for rainy season (Pratap *et al.* 2013; Gupta *et al.* 2001; Saxena and Yadav 1975). Sixty days maturing pulses including urdbean can fit in any cropping system thereby increase the cropping intensity. One urdbean genotype, IPU19-27, was developed at ICAR- Indian Institute of Pulses Research, Kanpur which mature significantly early (60-65 days) during summer

as well as rainy seasons as compared to other released urdbean varieties (70-80 days) in North-Eastern Plain Zone (NEPZ). This genotype was developed from the cross 'SPS 5 x IPU02-33' following the pedigree method of selection. This urdbean genotype showed resistance to mungbean yellow mosaic India virus (MYMIV) under high natural disease pressure conditions as evidenced in susceptible genotypes of urdbean. The major morphological characteristics of this newly developed genotype are medium height and erect plant type, light green lanceolate leaves, bright yellow flowers, medium length black pods on maturity with pubescence, and dull black seeds. This genotype has the potential to be released as a cultivar after multi-location testing and can also be a useful donor for earliness and MYMIV resistance.

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52. RKG-13-55 (IC0633092; INGR21052), a Chickpea (*Cicer arietinum*) Germplasm Resistant against Wilt. Good yield and Early

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The *desi* chickpea genotype RKG 13-55 has been developed by crossing of RSG 931 with RKG 143 at ARS, Kota in the year 2011-12 with the objective of combining high yield with disease resistance. It was tested in station trials and later evaluated in coordinated trials of AICRP on Chickpea across the zones in Initial Varietal Trial (IVT-Desi) in the year 2017-18. It was at par with the checks in yield but showed resistant reaction against fusarium wilt disease. Based on its resistant reaction, it was further evaluated for second year in 2018-19 in sickplots of plant pathological trials where it again showed the resistant reaction against wilt. The chickpea genotype RKG 13-55 was found resistant against wilt for consecutive two years (2017-18 and 2018-19) in Central and South zone; as per the results of the coordinated plant pathological trials of AICRP on Chickpea conducted in sick plots, as compared to checks GNG 2171, CSJ 515 and JG 315

Besides being resistant, RKG 13-55 also gave good yield, higher or at par with the leadind checks viz., GNG 1581, GNG 2171, JG 16, GCP 101, GCP 105, KWR 108 and JAKI 9218 (data enclosed). It was evaluated in Initial Varietal Trial (Desi) across the zones during the year 2017-18. In NWPZ, it yielded

2324 kg/ha which was at par with the checks GNG 1581 and GNG 2171 (2328 kg/ha and 2682 kg/ha, respectively). It yielded 1419 kg/ha in NEPZ which was at par with the checks KWR 108 and GCP 105 (1370 kg/ha and 1670 kg/ha, respectively). The genotype RKG 13-55 gave 2184 kg/ha yield in WCZ which was at par with the checks JG 16 and GCP 101 (2085 kg/ha and 2179 kg/ha). The genotype RKG 13-55 gave 1922 kg/ha yield in ECZ which was at par with the checks JG 16 and JAKI 9218 (1997 kg/ha and 1430 kg/ha). The genotype RKG 13-55 gave 1386 kg/ha yield in SZ which was at par with the checks JG 11 and JAKI 9218 (1865 kg/ha and 1833 kg/ha). The genotype RKG 13-55 matures in 139 days in North West Plain Zone, 130 days in North Eastern Plain Zone, 111 days in West Central Zone, 102 days in East Central Zone and 95 days in South Zone. It is early or at par with the checks GNG 1581, GNG 2171, JG 16, GCP 101, GCP 105 and KWR 108 in NWPZ, NEPZ, WCZ, ECZ and SZ (data enclosed). The genotype RKG 13-55 has brown, wrinkled, medium bold seed size. The 100-seed weight of RKG 13-55 was 24.2g in NWPZ, 23g in NEPZ, 23.2g in WCZ, 20g in ECZ and 24.8 g in SZ.

53. EC720481 (ILWC246) (EC720481; INGR21053), a Chickpea (*Cicer echinospermum*) Germplasm Resistant against Botrytis Gray Mold

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The wild chickpea accession EC720481 (ILWC246) was selected after preliminary characterization and evaluation of wild annual *Cicer* species for important agro- morphological and major biotic stress related traits during winter 2011-12

and summer 2012. All available global wild *Cicer* species were also evaluated against Ascochyta Blight, Botrytis Gray Mold and Root Knot Nematode, which had resulted into the identification of some resistant accessions against the target

Table 1: Descriptor and descriptor state of EC720481 (ILWC246) for important qualitative characters

Descriptor	Descriptor state
Plant pigmentation	Absent
Plant hairiness	Lightly pubescent
No. of leaflets leaf ¹	11-13
Flower colour	Light blue
Seed shape	Angular
Seed colour	Black
Testa texture	Tuberculated

traits. The highly resistant accession EC720481 (ILWC246) was further validated twice under controlled screening test using cut-twig screening technique, in which water was used as supportive medium. Twigs were inoculated by spraying spore suspension of *Botrytis cinerea* (10,000 spores ml⁻¹) and covered gently with moist chambers for 144 hrs. An incubation period of 8 hrs. dark and 16 hrs. light was provided with fluorescent lamps. Further observations on disease incidence were recorded using 1-9 rating scale. Based on the disease score, an accession EC720481

(ILWC246) belonging to *Cicer echinospermum* has been reported highly resistant against the BGM (Singh *et al.* 2014.).

Morpho-agronomic characteristics: Besides possessing resistance against Botrytis Gray Mold (BGM), an accession EC720481 (ILWC246) was also reported promising for other agro- morphological traits viz; number of branches/ plant (13), number of pods/ plant (31) and number of seeds/plant (33). As far as distinct morphological traits are concerned, the following qualitative features were also reported using chickpea descriptor states developed by IBPGR/ICARDA (Table 1).

The above mentioned important characters have their special significant value for enhancing genetic gains of cultivated varieties, which need to be considered while planning future chickpea genetic improvement programme for introgressing resistance against BGM as well as agronomic improvement of cultivated gene pool.

References

- IBPGR/ICARDA (1993) Chickpea descriptors: IBPGR Secretariat, Rome, Italy pp 15.
Singh *et al.* (2014) *Crop Science*, **54**:229-239.

54. EC720438 (ILWC229) (EC720438; INGR21054), a Chickpea (*Cicer reticulatum*) Germplasm Resistant to Ascochyta Blight

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The wild chickpea accession EC720438 (ILWC229) was selected after preliminary characterization and evaluation of wild annual *Cicer* species for important agro- morphological and major biotic stress related traits during winter 2011-12 and summer 2012. All available global wild *Cicer* species were also evaluated against Ascochyta Blight, Botrytis Gray Mold and Root Knot Nematode, which had resulted into the identification of some resistant accessions against the target traits. The highly tolerant accession EC720438 (ILWC229) was further validated twice under real filed hot spot at CSKHPKV Research and Extension Centre at Dhaulakuan. It was then artificially inoculated by frequently spraying with ascospore suspension (1x10⁶ spores ml⁻¹) using local isolates of *Ascochyta rabiei*. Further observations on disease incidence were recorded using 1-9 rating scale. Based on the disease score, an accession EC720438 (ILWC229) belonging to *Cicer reticulatum* has been reported highly resistant against the Ascochyta blight (Singh *et al.* 2014, *Crop Sci.*).

Morpho-agronomic characteristics: Besides possessing resistance against Ascochyta blight, an accession EC720438 (ILWC229) was also reported promising for other agro-morphological traits viz; number of branches/ plant (12), number of pods/ plant (37) and number of seeds/plant (39). As far as distinct morphological traits are concerned,

Table 1: Descriptor and descriptor state of EC720438 (ILWC229) for important qualitative characters

Descriptor	Descriptor state
Plant pigmentation	Low anthocyanin
Plant hairiness	Lightly pubescent
No. of leaflets leaf ¹	09-11
Flower colour	Light pink
Seed shape	Angular
Seed colour	Brown
Testa texture	Rough

the following qualitative features were also reported using chickpea descriptor states developed by IBPGR/ICARDA (Table 1).

The above mentioned important characters have their special significant value for enhancing genetic gains of cultivated varieties, which need to be considered while planning future chickpea genetic improvement programme

for introgressing resistance against Ascochyta blight as well as agronomic improvement of cultivated gene pool

References

- IBPGR/ICARDA (1993) Chickpea descriptors: IBPGR Secretariat, Rome, Italy pp 15.
Singh *et al.* (2014) *Crop Science*, **54**:229-239.

55. IC259504 (IC0259504; INGR21055), a Wild Bean (*Vigna vexillata*) Germplasm High protein content (9.5%) in tuber. Bold seededness. Fodder type.

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Tuber cowpea, *Vigna vexillata* (L.) A. Rich. is an underutilized tuberous resilient legume which is known for its multiple uses in India. Being a leguminous tuber, protein is the most important useful trait for enhancing utilization of this crop. A total of 108 accessions of *V. vexillata* were obtained in the form of seed from the Indian national genebank, ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi. After screening for agronomic and biochemical traits, the accession IC259504 was found with more than 9.5% protein content in tuber.

Besides high protein content, this accession also has superior agronomic traits *viz.* tuber weight, pod length, number of seed per pod and bold seededness (5.01g). Nutritional compositions were also compared with other major tuber crops *viz.*, sweet potato, cassava and soh-phlong, a minor leguminous tuber from India. In comparison to other crops, tuber cowpea was found to have seven-fold and nine-fold higher amount of protein content than sweet potato and cassava respectively but threefold to soh-phlong.

Sasikumar and Sardana (1988) reported proximate compositions on seeds of tuber cowpea, but they did not give any information on tubers. This is the first study allows a glimpse into the variability in tubers of the Indian region. *V. vexillata* is widely distributed in all four biodiversity hotspots designated in India. Despite low variability in tubers reported globally, this investigation revealed sufficient variation for tuber morphology, nutritional traits and habitat study. Utilization of this underutilized legume can lead to improvement in tuber cowpea as a root crop besides pulse, vegetable and forage crop.

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56. IPC HT2A & IPC HT2B (IC0635034 & IC0635035; INGR21056), a Carrot (*Daucus carota*) CMS line IPC HT2A is First Red Colour Heat Tolerant Tropical Carrot Developed Indigenously. Roots are of Acceptable Size, Red Colour and Self Core. Suitable for Early Season Sowing due to its Pusa Vrishti (IPC HT2) Genotype Background.

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Carrot (*Daucus carota* L.; 2n= 2x=18) is important nutritious root crop. It is being grown all across the world on an area of 1.15 million ha area with production of 42.8 million tonnes (FAOSTAT, 2017). It has temperate (European) and tropical (Asiatic) types. In India, it is being grown on 86,200 ha area

with a production of 1,379,031 MT (NHB Database 2017). Here, both tropical and temperate types are popular but tropical red carrot are predominant due to their suitability for juice, *halwa*, sweets and vegetable purposes especially in north India. However, productivity of carrot in India is

around 33.5% lower than world average mainly due to lack of hybrids which are known for uniformity, earliness, high yield, and more percentage of marketable roots (Kalia *et al.* 2019). In carrot, the cytoplasmic male sterility (CMS) is being used commercially for hybrid breeding which is of two types *i.e.* petaloid (anthers turns to petals) and brown anther (shriveled brown anther). Although, both systems are in use for commercially hybrid breeding seed production but petaloid type is environmentally stable hence, widely used in hybrid breeding of carrot. Presently, the only hybrid available in tropical red carrot is Pusa Vasuda which is developed by use of petaloid CMS system and suitable for main season cultivation only. However, demand is also increasing for early season carrots and conversion of elite inbred lines of early season having heat tolerance with stable CMS was required for early season hybrid breeding. The CMS line IPC HT2A is an indigenously developed heat tolerant CMS line of tropical carrot. It has petaloid type sterile cytoplasm into the genetic background of an elite inbred line IPC HT2B which is suitable for early season July sowing in the north Indian plains because of heat tolerance. The IPC HT2 (fertile) has been converted to IPC HT2A (male sterile) by introgression of petaloid CMS system from 'IPC 122A', a natural mutant identified at Division of Vegetable Science, ICAR-IARI, New Delhi (Kalia *et al.*, 2019) and backcrossing with recurrent parent (*i.e.* IPC HT2) for nine generations (BC₉).

Morpho-agronomic characteristics: The CMS line IPC HT2A is the first CMS line of tropical heat tolerant red carrot developed indigenously. It is suitable for sowing during early crop season (*i.e.* July end to mid August) and roots are ready to harvest in a period of 90 – 95 days (Table 1). The height of flowering plants was 129.6 cm with profuse flowering umbels and seed setting. The floral traits *i.e.* petal size, petal colour, petaloid (anther converted petals) colour and size and nectaries showed normal development (Fig. 1A-B; 2A-B). It produces red colour, medium long roots with self-core character. The average root length was observed to be 21.5 cm, root diameter 32.3 mm, core diameter 7.86 mm and root weight 100 g (Fig. 3A-B). The marketable root yield was observed to be 15.0 t/ha which was significantly low than its maintainer line IPC HT2B (27.5 t/ha) (Table 1). The bolting (elongation of flower stalk) occurs during January-February month and produce abundant seeds during March-April month in plains of India. The CMS line showed potential in hybrid breeding of early season carrot hybrid with tolerance to heat stress hence, can be used in commercial hybrid breeding programme.

The 'IPC HT-2B' is a promising genotype of early season tropical carrot with heat tolerance trait. It is suitable for sowing in July end to August first fortnight in north Indian plains. It has been already released as 'Pusa Vrishti' for commercial cultivation. It gets ready for harvesting in

Table 1: Important horticultural traits and seed yield of CMS line IPC HT-2A line as compared to the fertile maintainer line IPC HT2B

Traits	IPC HT-2A	IPC HT 2B
Maturity traits		
Growing season	Early season	Early season
Heat tolerance	Yes	Yes
Sowing period	July end to August first fortnight	July end to August first fortnight
Harvesting	October end to November first fortnight	October end to November first fortnight
Plant and root traits		
Plant height (cm)	78	85.2
Gross plant weight (cm)	185	215.0
Root colour	Red	Red
Root core colour	Red	Red
Root length (cm)	21.5	20.4
Root diameter (mm)	32.3	37.9
Core diameter (mm)	7.86	7.14
Root weight (g)	100	150
Root yield (t/ha)	150	275
Floral traits		
Petal colour	White	Light green
Petal length (cm)	0.99	0.44
Petaloid colour	Light purple	-
Petaloid length (cm)	1.67	-
Petaloid width (cm)	0.62	-
Petaloid shape	Spoon	-

90-95 days during last week of October to first fortnight of November. Plants are medium vigorous, semi-erect, medium in spread and leaves are green. It produces red root with self core colour (Table 1).

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57. BR 161 (IC0637585; INGR21057), a Cauliflower (*Brassica oleracea* var. *botrytis*) Germplasm Resistant to Black Rot Disease (*Xanthomonas campestris* pv. *campestris* race 1). Carries a Novel Single Dominant Gene *Xca1bo* for Black Rot Resistance Located on Chromosome 3 and Flanked by DNA Markers

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Cauliflower (*Brassica oleracea* var. *botrytis* L.) is one of the most important and widely grown *Brassica* vegetables in India. Among the diseases, black rot is a wide spread bacterial disease caused by *Xanthomonas campestris* pv. *campestris* (Xcc) (Pammel) Dowson. It is the most devastating disease of cauliflower worldwide including India (Singh and Dhar, 2011; Saha *et al.*, 2014). The line 'BR 161' is resistant to black rot disease caused by Xcc race 1. It was developed from progeny selection of a cross of S. No. 15 (susceptible genotype) and MGS-2-3 (resistant) by Division of Vegetable Science, ICAR-IARI, New Delhi, India. The line carries a novel single dominant resistant gene *Xca1bo* on chromosome 3 and flanked by DNA markers RAPD 04₈₃₃ and ISSR 11₆₃₅ (Saha *et al.*, 2014).

Morpho-agronomic characteristics: The resistant line BR 161 belongs to mid-late maturity group with erect growth habit and medium in height (48–50 cm). The leaves are light green, medium in size with weak puckering habit. The curds are open, loose and creamy with an average weight of 401.05 g. The reaction of the plant at adult stage showed resistant reaction with plant disease index of 0-0.4 even at 60 days after inoculation (DAI) (Table 1) (Saha *et al.*, 2012).

Associated characters and cultivation practices: The curd

of the line BR 161 become mature in the month of December end to mid- January requiring 12-16°C temperature for curd initiation and development. The crop gets ready to harvest in 75-80 days after transplanting in its normal growing season. The flowers of 'BR 161' are yellow and stalk length is medium and flowering occurs during February end to mid-March. The line can be a useful source for transferring the resistant gene in commercially susceptible variety (s) or to develop hybrid (s).

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Table 1: Response of BR 161 and commercial cauliflower varieties to black rot disease in four different season/environment after artificial inoculation with black rot pathogen (Xcc race 1)

Genotype/lines	PDI at 60 days after inoculation Environment 1/season 1 (a)	PDI at 60 days after inoculation Environment 2/season 2 (b)	PDI at 60 days after inoculation Environment 3/season 3 (c)	PDI at 60 days after inoculation Environment 4/season 4 (d)	Disease reaction*
BR 161**	0.2	0.1	0.4	0.0	Resistant
Pusa Himjyoti	3.9	4.0	4.0	4.0	Highly susceptible
Pusa Sharad	3.4	3.9	3.8	4.0	Highly susceptible

*Based on PDI score developed by Thakur *et al.* (2003), 0-1: Resistant; 1-2: Moderately resistant; 2-3 Susceptible; 3-4: Highly susceptible.

58. DSG-7 (IC0588957) (IC0588957; INGR21058), a Sponge Gourd (*Luffa cylindrica*) Germplasm Highly Resistant to Tomato Leaf Curl New Delhi Virus. Good combiner and Gives Higher Heterosis for Yield & other Desirable Traits. Resistance is Governed by Single Dominant Gene.

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Sponge gourd is a popular low cost vegetable grown throughout India. Recently *Tomato Leaf Curl New Delhi Virus*, (ToLCNDV: genus *Begomovirus*, family Geminiviridae), the causal virus of tomato leaf curl has been reported to be associated with sponge gourd and causing a yield loss up to 100 % in north Indian plains during *kharif* season under epidemic condition (Sohrab *et al.*, 2003). The diseased plant is characterized by yellow spots appearing on newly emerging leaves, followed by a mosaic appearance and upward curling of the upper leaves. In cases of severe attack, the leaves of the plant are small and distorted and misshapen fruits are produced. The virus is transmitted through sap as well as whitefly (*Bemisia tabaci* Hemiptera-Aleyrodidae). The genotype DSG-7 was screened under natural epidemic condition and found to be completely resistant to *Tomato leaf curl New Delhi virus*, during rainy season which is conducive for white fly multiplication and the spread of diseases. The result was also confirmed through challenge inoculation with purified strain of the virus under insect

proof green house at Virology Unit, Division of Plant Pathology, IARI, New Delhi.

DSG-7 is developed by selection from segregating material collected from Moradabad district of Uttar Pradesh. It is a monoecious trailing or climbing annual vine with stem and tendrils. The leaves are medium green, reniform, six lobed, nearly glabrous, non-hairy and smooth with long petioles. Stem is angular. Inflorescence is clustered, racemose, flowers unisexual, male flower large in number in cluster, yellow and showy with long peduncle. Female flower is solitary on short and round peduncle and yellow in colour. Fruits elongated (15-20 cm), straight, light green with thin skin and without stripes, average fruit weight 110g, flesh tender, suitable for spring-summer and *kharif* season. It is ready for first harvesting in 45-50 days during *kharif* season and 50-55 days in spring summer season. Average fruit yield is 16 t/ha. Highly resistant to *Tomato leaf curl New Delhi virus* during *kharif* season which is conducive for white fly multiplication and the spread of diseases (Table 1 to 5).

Table 1: Screening of DSG-7 under natural epidemic condition during *kharif* season 2008-2010

Genotype	2008		2009		2010	
	Yield (t/ha)	Vulnerability Index & Category	Yield (t/ha)	Vulnerability Index & Category	Yield (t/ha)	Vulnerability Index & Category
DSG-7	13.95	8.67 (HR)	14.56	4.15 (HR)	14.05	6.67 (HR)
CHSG-1	1.65	100.00 (HS)	2.16	98.48 (HS)	2.15	97.50 (HS)
CHSG-2	2.01	99.33 (HS)	2.25	96.86 (HS)	2.34	95.25 (HS)
PSG-9	1.84	99.33 (HS)	2.39	95.10 (HS)	2.03	97.55 (HS)
KG-3/134 (IC 284787)	1.92	98.00 (HS)	1.85	99.05 (HS)	2.07	95.88 (HS)

HS: Highly susceptible; HR: Highly Resistant

Table 2: Screening of DSG-7 for resistance to *Tomato leaf curl New Delhi virus* through challenge inoculation (*Bemisia tabaci*) under greenhouse condition during 2008

Genotype	Source	Vulnerability Index	Category
DSG-7	IARI, New Delhi	6.00	Highly Resistant
CHSG-1	HARP, Ranchi	100.00	Highly susceptible
CHSG-2	HARP, Ranchi	100.00	Highly susceptible
PSG-9	PAU, Ludhiana	99.33	Highly susceptible
KG-3/134 (IC 284787)	NBPGR, New Delhi	98.67	Highly susceptible

Table 3: Screening of DSG-7 under natural epidemic condition during *kharif* season 2011-2013

Genotype	2011		2012		2013	
	Yield(t/ha)	Vulnerability Index & Category	Yield(t/ha)	Vulnerability Index & Category	Yield (t/ha)	Vulnerability Index & Category
DSG-7	14.30	8.90 (HR)	14.75	6.30 (HR)	15.00	6.85 (HR)
CHSG-1	1.90	100.00 (HS)	2.28	99.33 (HS)	2.35	98.48 (HS)
CHSG-2	2.15	98.00 (HS)	2.50	100.00 (HS)	2.10	96.25 (HS)
PSG-9	2.00	96.86 (HS)	2.45	97.30 (HS)	2.20	98.55 (HS)
KG-3/134 (IC 284787)	1.98	98.48 (HS)	1.90	99.15 (HS)	2.00	96.80 (HS)

HS: Highly susceptible; HR: Highly Resistant

Table 4: Screening of DSG-7 under natural epidemic condition during *kharif* season 2014-2016

Genotype	2014		2015		2016	
	Yield(t/ha)	Vulnerability Index & Category	Yield(t/ha)	Vulnerability Index & Category	Yield (t/ha)	Vulnerability Index & Category
DSG-7	15.20	7.50 (HR)	14.60	8.75 (HR)	14.85	7.20 (HR)
CHSG-1	2.10	99.33 (HS)	2.40	97.25 (HS)	2.05	100.00 (HS)
CHSG-2	2.25	99.33 (HS)	2.30	98.50 (HS)	2.50	98.48 (HS)
PSG-9	1.75	97.50 (HS)	2.04	97.10 (HS)	1.98	95.90 (HS)
KG-3/134 (IC 284787)	2.00	98.00 (HS)	1.95	98.60 (HS)	1.85	96.75 (HS)

HS: Highly susceptible; HR: Highly Resistant

Table 5: Screening of DSG-7 under natural epidemic condition during *kharif* season 2017-2019

Genotype	2017		2018		2019		Average yield (t/ha)
	Yield(t/ha)	Vulnerability Index & Category	Yield(t/ha)	Vulnerability Index & Category	Yield(t/ha)	Vulnerability Index & Category	
DSG-7	14.72	3.80 (HR)	17.30	3.30 (HR)	16.40	3.30 (HR)	16.14
DSG-6	11.23	4.30 (HR)	16.90	4.10 (HR)	12.70	4.30 (HR)	
Pusa Supriya (check)	5.89	89.20 (HS)	4.20	89.30 (HS)	4.80	89.20 (HS)	
Kalyanpur							
Hari Chikni(check)	4.45	94.30 (HS)	4.65	84.90 (HS)	4.10	94.30 (HS)	

HS: Highly susceptible; HR: Highly Resistant

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59. DPMFWR-30 (IC0637586; INGR21059), a Fasciation Plant type Pea (*Pisum sativum*) Germplasm with. Synchronized Flowering and Pod Formation. Putative Mutant Synthesised from Azad P-1.

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Genotype	Days to flowering	Days to first picking	Pod length (cm)	Pod width (cm)	Seeds/pod	Shelling (%)	Plant height (cm)	Pods/plant	Average pod weight (g)	Pod yield/plant (g)
DPPMFWR-30	96.33	141.33	8.95	1.69	5.53	45.50	61.80	10.23	3.75	37.73
Azad P-1 (c)	91.33	143.67	9.59	1.82	6.27	47.57	92.20	19.08	4.04	76.94
Palam Priya (c)	88.00	132.67	8.64	1.61	6.27	45.73	70.82	14.43	3.38	48.76

For processing industry, it is expected to harvest peas once only but it exacts a compromise of both yield and quality. At harvest stage, the pods on the plant are not matured concurrently and are of variable size and tenderness. Despite the customary attempt to select a harvest date consistent with maximum returns of prime quality pea, some of the pods are outside the limits of optimum quality either overmature or too immature. There are two sources of variation that contribute to the overall range of maturity experienced in the field. The first, interplant variation involves relative differences in maturity among neighbouring plants which are associated with cultural practices and local environmental conditions. The second one is intraplane variation, involves progressive maturity on the plant.

The garden pea is characterized by a compound racemose inflorescence. The main shoot proliferates unlimitedly during whole vegetation period and produces frondose leaves subtending short bractless axillary racemes usually of one or two flowers. The commercial varieties of pea are characterized by the production of inflorescences in successive leaf axils. Each succeeding inflorescence being less mature than the preceding ones. Frequently, both inflated pods and unopened flowers may be observed on the same plant. This variation is subject to genetic control. Fasciation is associated with genetic mutation (Lerner, 2007) and is significant for uniform maturity and mechanical harvesting. Breeding programmes established based on the variability induced by mutagenic treatment are commonly used for improvement of the most productive and well-adapted varieties (Kalapchieva and Tomlekova 2016).

Morpho-agronomic characteristics: Induced mutations have played a significant role in meeting challenges

related to world food and nutritional security by way of mutant germplasm enhancement and their utilization for the development of new mutant varieties. Different morphological putative mutants were synthesised. The fasciated mutant was obtained from Azad P-1 variety treated with EMS @ 0.3%. The fasciation in pea give the plant a witches-broom appearance based on arrangement and order of both foliar and floral leaves (Marx and Hagedorn 1962) which is accompanied with a progressively dilated, twisted stem axis. The distinctive characteristic of fasciated individuals is having shorter shoots compared to control cultivars due to shortening of upper internodes that led to appearance of flowers and pods from a pseudo umbel/false umbel. The most desirable fasciated plants would be the one that have similarity to that of normal pea plant except for the position of the pods. It is the concentrated pod set that is attractive from the viewpoint of uniform maturity since the inflorescences usually are borne over a relatively short period of time. The line was evaluated in 2011-12 and in subsequent years. Its yield is comparatively low from the check varieties but has synchronized maturity. It is important to introgressed this trait of interest in different varieties to synthesized genotypes with synchronized maturity vis-à-vis pod characteristics as per consumers' preference and high yield.

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60. DGRMB 5 (IC0637587; INGR21060), a Groundnut (*Arachis hypogaea*) Germplasm Tolerant to Salinity. CAM (Crassulacean Acid Metabolism) Variant.

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The groundnut cultivated in India (area: 4.92 mha; production: 10 MT; productivity: 2065 kg/ha) is susceptible to both drought- and salinity- stress. Thus, efforts are being made to develop groundnut varieties tolerant to drought- and or salinity- stress following both conventional breeding and transgenic approaches, but with limited success. Therefore, transferring entire genetic machineries of CAM (Crassulacean Acid Metabolism; a water saving mechanism) pathways of desert succulents into C_3 plants, is considered an option for imparting drought-tolerance (Davis *et al.* 2015). Moreover, facultative CAM is also reported to impart salinity tolerance in *Mesembryanthemum crystallinum* (Winter and Holtum 2014). However, salinity- tolerant cultivars through CAM transition are yet to be identified in any field crops of agricultural importance.

The variants were selected from groundnut cultivar TG 37A by imposing drought (no water after emergence till harvest). Twelve such selections were evaluated for salinity- tolerance in addition to expression of all modules (carboxylation, decarboxylation, mesophyll succulence, and invert stomatal behaviour) of facultative CAM and quantified (ap Rees *et al.* 1983; Merlo *et al.* 1993) in field during summer seasons for salinity. DGRMB 5 was evaluated for salinity tolerance for three seasons (upto av. 4.5 soil EC at harvest during summer of 2017, 2018 and 2019) at Junagadh and one season (kharif 2019) at 4.55 soil EC at the CCSRI, Mandvi, Bhuj, Gujarat.

DGRMB 5 is a Spanish bunch, salinity- tolerant facultative CAM-photosynthetic transitioned variant of TG37A, obtained through selection at the ICAR-Directorate of Groundnut Research, Junagadh, Gujarat. In addition, it has expressed all modules of facultative CAM under salinity-stress as a tool to impart tolerance. It differed genetically from TG 37A, TG

38, TAG 24, TG 51, TG 26 and among other variants on the basis of genomic and EST based SSR markers: three genomic (PM137, pGPseq18E7 and pGPseq2G4) and four EST (DGR114, DGR289, DGR811 and DGR2474). It has moderate plant height, irregular branching with flower on main axis, and small dark green lanceolate leaves. It bears compound inflorescence with light yellow coloured standard petal and takes about 37 days after sowing for 50% flowering in salinity stress. It produced ~156 g of pod/m²; ~32% of Harvest Index, ~65% shelling out-turn and ~37 g of HKM. In salinity condition, kernels of DGRMB 5 are having tan colour and mature in 98 days after sowing. DGRMB 5 can be cultivated directly as a salinity- tolerant (upto 4.5 soil EC at harvest) genotype or as a donor for imparting salinity- tolerance through CAM-photosynthetic transition in other genotypes. Salinity- tolerance through facultative CAM- transition is the first report in any agricultural crop.

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61. DGRMB 19 (IC0637588; INGR21061), a Groundnut (*Arachis hypogaea*) Germplasm Tolerant to Salinity.

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Even through in India, groundnut (*Arachis hypogaea* L.) is cultivated in about 4.9 mha with production of around 10.0 MT with productivity of 2065 kg/ha, it is susceptible to both drought- and salinity- stress. Hence, efforts are being made to develop groundnut varieties tolerant to drought- and or salinity- stress, but with limited success employing both conventional and transgenic approaches. Therefore, transferring entire genetic machineries of CAM (Crassulacean Acid Metabolism) pathways of desert succulents into C_3 plants, is considered an option for imparting drought-tolerance (Davis *et al.* 2015). Moreover,

facultative CAM is also reported to impart salinity tolerance in *Mesembryanthemum crystallinum* (Winter and Holtum 2014). However, salinity- tolerant cultivars through CAM transition are yet to be identified in field crops of agricultural importance.

The variants were selected from TG 37A by imposing drought (no water after emergence till harvest). Selections were also characterised for salinity- tolerance. In addition, they were characterised for expression of all modules (carboxylation, decarboxylation, mesophyll succulence, and invert stomatal behaviour) of facultative CAM and

quantified (ap Rees *et al.* 1983; Merlo *et al.* 1993) in field during summer seasons. DGRMB19 was evaluated for three seasons for salinity tolerance (upto av. 4.5 soil EC at harvest during summer of 2017, 2018 and 2019) at Junagadh and one season (*kharif* 2019 at soil EC 4.55) at the CCSRI, Mandvi, Bhuj.

DGRMB 19 is a Spanish bunch, salinity- tolerant facultative CAM-photosynthetic transited variant, selected from TG 37A, at the ICAR-Directorate of Groundnut Research, Junagadh, Gujarat. In addition, it has expressed all modules of facultative CAM under salinity stress as a tool to tolerance. It differed genetically from TG 37A, TG 38, TAG 24, TG 51, and TG 26 on the basis of genomic (one: PM137) and EST (two: DGR114 and DGR811) based SSR markers. It has moderate plant height, irregular branching with flower on main axis and small dark green oblong-lanceolate leaves. It bears compound inflorescence with dark yellow coloured standard petal and takes about 38 days after sowing for 50% flowering in salinity stress. It produced ~151 g of pod/m²; ~31% of Harvest Index; ~64% shelling out-turn; and ~36 g of 100-kernel weight. In salinity- condition, kernels of DGRMB 19 are having light tan in colour and matures in 100

days after sowing. DGRMB 19 can be cultivated directly as a salinity- tolerant (upto av. 4.5 soil EC at harvest) genotype or as a donor for imparting salinity- tolerance through CAM transition in other genotypes. This is a unique source of salinity- tolerance through CAM-photosynthetic transition.

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62. DRMRQ1-16-27 (IC00637589; INGR21062), an Indian mustard (*Brassica juncea*) Germplasm with High Antioxidants (Phenol and Tocopherol). Low Antinutritional Component (Phytic Acid). Double Low (<2% Erucic Acid in Oil and <30 µmoles Glucosinolate/G Defatted Seed Meal).

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The strain (DRMRQ1-16-27) is a double low Indian mustard line possessing high level of antioxidants (phenol content; 5.16% and tocopherol content; 196.93mg/100g seed meal) with low level of anti-nutritional components (phytic acid content; 1.78% and glucosinolate content; <13.74 µmoles/g of defatted seed meal, erucic acid; <2%) both in oil and seed meal. The strain namely, DRMRQ1-16-27 was selected as single plant selection following pedigree breeding method from the cross EC564648 x (NUDHYJ-3 x PCR-7). The strain (DRMRQ1-16-27) evaluated in the year 2018-19 at DRMR along with checks both morphologically in the field and biochemically. It was found to have low erucic acid content (<2% in oil) comparable to low erucic quality check (PM21), and also low glucosinolate content (<30µmoles/g of defatted seed meal) comparable to double low quality check (PDZ1 and RLC3). The strain (DRMRQ1-16-27) was further analyzed biochemically for quality traits including erucic acid, glucosinolate, beta carotene, antioxidants, tocopherol, phenol and phytic acid in the year 2019-20 at DRMR (Table 1)

DRMRQ1-16-27 was also evaluated in biochemistry trials of AICRP-RM programme 2018-19 at six independent centers namely Bharatpur, Kanpur, Kangra, Pantnagar, Hisar and Ludhiana for antioxidant properties (phenol content and tocopherol content) and anti-nutritional factors (phytic acid and glucosinolate). The strain, DRMRQ1-16-27 showed highest phenol content (5.16%) and tocopherol content (196.93mg/100g seed meal) among 22 tested entries along with quality check, PDZ1 (phenol content (4.26%) and tocopherol content (162.39mg/100g seed meal). The strain, DRMRQ1-16-27 showed lowest phytic acid content (1.78%) and glucosinolate content (<13.74 µmoles/g of defatted seed meal) among 22 tested entries along with quality check, PDZ1 (phytic acid content (2.61%) and glucosinolate content (<21.11 µmoles/g of defatted seed meal).

The strain, DRMRQ1-16-27 have been registered as a germplasm, because of having high level of antioxidants and low level of anti-nutritional compound in oil and seed meal. The strain can act also as a check in AICRP-RM biochemistry trials and donor for the quality traits.

Table 1: Comparative data of quality traits of strain (DRMRQ1-16-27) and checks

Genotypes/Traits	Erucic Acid (%)	Glucosinolate (μ moles/g)	Beta carotene (ppm)	Antioxidant (mg/g AAE)	Total phenol (%)	Tocopherol (mg/100g)	Phytic acid (%)
DRMRQ1-16-27	1.9	19.3	3.98	38.85	6.58	197.52	1.62
DRMRQ 4-7-23	1.5	15.9	2.74	35.35	5.48	182.50	2.16
PDZ1 (Double low quality check)	1.65	22.6	2.77	29.35	4.45	162.98	2.85
NRCHB 101 (Non quality check)	39.38	136.7	3.26	47.35	3.59	147.58	3.36

63. DRMRIJ 12-40 (IC0637590; INGR21063), an Indian mustard (*Brassica juncea*) Germplasm Resistant to White Rust Disease. Presence of Two Different Genes Conferring Resistance against White Rust. Good Agronomic Base.

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White rust caused by *Albugo candida* (Pers. ex Lev.) Kuntze is one of the most devastating diseases of Crucifers and causes severe losses in Indian mustard (*Brassica juncea*). Genetic studies have revealed dominance of resistance over susceptibility. Resistance to white rust has been shown to be governed by a single dominant gene.

DRMRIJ 12-40 is an inbred line derived from a cross between Zem 2 (exotic germplasm) and JGM 1-11 followed by individual plant selection. Cross was attempted during 2004-05 between Zem 2 and JGM 1-11. F_1 generation was raised at Bharatpur during 2005-06 and F_2 generation was raised at IARI Regional Station Wellington during 2006. Homozygous single plants were selected from homogenous resistant lines. F_3 - F_4 generations were raised at Bharatpur and Wellington, respectively. Subsequent generations were raised at Bharatpur. Single plants were selfed and advanced from F_6 (2008-09) to F_8 (2010-11) generation. In 2011-12 promising resistant line was named as DRMRIJ 12-40 and later on maintained through selfing. DRMRIJ 12-40 was inducted to AICRP-RM during 2015-16 and had been evaluated continuously for four years during 2015-16, 2016-17, 2017-18 and 2018-19. White rust reaction at leaves was recorded at two different stages viz., 75 and 100 days, while staghead formation was recorded at flowering stage. Candidate strain DRMRIJ 12-40 expressed resistant reaction against white rust at 75 days stage which was at par with

resistant check BIO YSR during two years of testing at Hisar. Mean

Disease index of DRMRIJ 12-40 was 0.0 in comparison to 34.1 of susceptible check Rohini. Disease reaction against white rust on leaves at 100 days stage was recorded at five locations viz., Hisar, Kangra, Kanpur, Pantnagar and Morena, which included three hot spots (Kangra, Pantnagar and Morena) (Table 1). On the basis of mean disease index over all five locations for four years, candidate strain DRMRIJ 12-40 expressed 2.45 % which was better than the resistant check BIO YSR (8.41%) and susceptible check Rohini (34.0%). Observations on staghead formation were recorded at Morena and on the basis of mean disease index over three years, DRMRIJ 12-40 had 0.0 % disease incidence in comparison to resistant check, BIO YSR (9.9%) and susceptible check Rohini (14.2%). Resistant reaction against white rust was confirmed with already reported IP markers and the candidate strain was found to possess both the WR resistance loci (Fig. 1). Hence, on the basis of disease reaction at five locations over four years and confirmation of resistance with molecular markers, candidate strain DRMRIJ 12-40 was found resistant against white rust.

Agronomic characterization of DRMRIJ 12-40: Strain DRMRIJ 12-40 was characterized for agronomic traits viz., days to flower initiation (52 days), plant height (205 cm), length of main raceme (73 cm), 1000-seed weight (4.1 g), siliquae length (5.5 cm).

Table 1: Mean disease index of candidate strain DRMRIJ 12-40 and check genotypes against white rust pathogen

S. No.	Location	White rust (% WR severity)								
		Disease reaction at 75 days			Disease reaction at 100days			Staghead formation		
		DRMR IJ 12-40	Bio YSR (Res. Check)	Rohini (Sus. Check)	DRMR IJ 12-40	Bio YSR (Res. Check)	Rohini (Sus. Check)	DRM RIJ 12-40	Bio YSR (Res. Check)	Rohini (Sus. Check)
										k)
1	Hisar	0.0	0.0	34.15	2.2	0	34.8			
2	Kangra				1.1	14.2	44.8			
3	Kanpur				0	0	31.7			
4	Pantnagar				5.5	9.4	29.0			
5	Morena				0.5	8.7	31.2	0.0	9.9	14.2
Mean		0.0	0.0	34.15	2.45	8.41	34.0		9.9	14.2

64. RG-3060 (IC0449033; INGR21064), a Castor (*Ricinus communis*) Germplasm with Resistance to Leaf Hopper

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Castor (*Ricinus communis* L.) is an important industrial oilseed crop. India is the top castor oil producing country. Castor crop suffers from several disease and insect attacks. Leafhopper *Empoasca flavescens* Fabr. (Cicadellidae: Hemiptera) is one of the major insect pests of castor causing yield loss up to 89% (Lakshminarayana and Duraimurugan, 2014). Cultivation of resistant cultivar is the best leafhopper controlling measure. For breeding a resistant cultivar, availability of resistance donor is a prerequisite. Diverse source of resistance is required for sustaining high yields in castor crop. Intensive search for leafhopper resistance was taken up at the ICAR-Indian Institute of Oilseeds Research, Hyderabad, India. This has led to identification of leafhopper resistant castor accession, RG-3060. It was developed through mass selection from a heterogeneous and heterozygous population of a wild collection collected from Morgrdh-1, Anjar (Taluque), Kutch, Gujarat (Anjani, 2012). RG-3060 was screened against leafhopper using infester-row method at three locations along with susceptible (DCH-177) and resistant (DCH-519) checks for three years. It has consistently exhibited resistance against leafhopper at all locations in all years of screening (Table 1).

Morpho-agronomic characteristics: RG-3060 has green stem with triple bloom, and green, spiny non-dehiscent capsules. It had 16-17 stem nodes, 30.7 g 100-seed weight and 48% oil content. RG-3060 flowers in 55-60 days and matures in 120-125 days.

Associated characters and utility: RG-3060 has also showed resistance (14-15% wilt incidence) against *Fusarium wilt* (*F. oxysporum* fsp. *ricini*) in wilt sick plots at two locations, while the susceptible check had 94-98% wilt incidence. RG-3060 is diverse from previously identified resistant sources as it is derived from a genetically diverse source. It would serve as a diverse source of leafhopper resistance in resistance breeding as well to tag/identify leafhopper resistant candidate gene.

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Table 1: Reaction of RG-3060 against leafhopper at multilocations in different years

Genotype	Year of screening	Palem		Yethapur		IIOR, Hyderabad		Reference
		LH no.	HPB (0-4 scale)	LH no.	HPB (0-4 scale)	LH no.	HPB (0-4 scale)	
RG-3060	2013-14	20.4	1.0	1.9	0.0	18.3	1.0	AICRP-Castor Annual Report- 2013-14, page No.205,206
DCH-177 (SC)		37.4	4.0	9.3	2.0	152.4	4.0	
DCH-519 (RC)		15.0	1.0	4.6	0.0	25.5	1.0	
RG-3060	2014-15	13.6	0.0	6.0	1.0	10.8	1.0	AICRP-Castor Annual Report- 2014-15, page No.179,187
DCH-177 (SC)		31.8	4.0	22.3	4.0	84.3	4.0	
DCH-519 (RC)		11.3	0.0	2.7	0.0	12.5	1.0	
RG-3060	2015-16	12.1	1	1.7	0.0	10.0	1.0	AICRP-Castor Annual Report- 2015-16, page No.200,201
DCH-177 (SC)		61.1	3.0	38.7	3.0	54.9	4.0	
DCH-519 (RC)		22.9	1.0	9.0	0.0	16.0	1.0	

SC: susceptible check; RC: resistant check; LH no.: no. of leafhoppers/3 leaves/plant; HPB: hopper burn

65. HOSuS-1 (IC0637591; INGR21065), a Sunflower (*Helianthus annuus*) Germplasm with High Oil Content (41%).

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Sunflower (*Helianthus annuus* L.) is native to North America and contains 52 species (67 total taxa) (Kane *et al.* 2013), occupying prime position in Indian economy. In India sunflower is introduced in 1969 as an oilseed crop, prior to which it was used as an ornamental plant. It is mainly grown for its oil which is used for culinary purpose in the preparation of *vanaspathi* and in the manufacture of soaps and cosmetics. Till date a total of 29 hybrids were released in sunflower suitable for different agro-climatic situations in India (Sujatha *et al.* 2019). Development of high oil content parental lines and hybrids are the major priority in India. In view of this and great prospect of cultivation of this crop successfully in various region of India, it is necessary to develop high oil yielding varieties/hybrids for different locations. HOSuS-1 is a selection from segregating progenies of the germplasm accession GP4-1424 of cultivated sunflower. This newly developed genetic stock will be very useful in developing high oil content and high oil yielding hybrids in sunflower.

Morpho-agronomic characteristics: Newly developed line HOSuS-1 was promising for oil content recording >40% consistently (Anonymous, 2018-19). The crop was raised in augmented block design along with checks and oil content was analysed through NMR during the year 2015, 2018, 2019 and 2020, respectively at ICAR-Indian

Institute of Oilseeds Research, Rajendranagar, Hyderabad and confirmed through Soxhlet. The average oil content of this line was 41.32% compared to high oil content check RHA-6D-1 (37.9%). The plants were unbranched, medium in maturity (83 days), medium in plant height (105 cm) with slender stems. Leaves and stems were smooth and non-pubescent. Leaves were large and green with triangular shape. Medium blistering was present on leaves without petiole pigmentation. The stem pigmentation was absent. The ray florets were many in number with elongated shape. The ray floret colour was yellow. The disc floret colour was orange without pigmentation. The head attitude of the line was half-turned down with flat shape and small size (11.4 cm). The bract shape of the genotype was elongated without pigmentation. Seed base colour was black with ovoid elongated shape. Average seed yield of the line was 24.0 g/plant with test weight of 3.9g and volume weight 44.0 g. Average oil content of the line was 41.32% (Table 1).

Table 1: Oil content data of HOSuS-1 through NMR and Soxhlet methods

Method	HOSuS-1	RHA 6D-1/DRSH-1 (Check)
NMR*	41.32	35.54
Soxhlet	41.91	37.75

*Mean of 4 years

Morpho-agronomic characteristics of HOSuS-1

Characteristics	Description
Plant height (cm)	105.0
Head diameter (cm)	11.4
Days to flowering	53
Days to maturity	83
Seed colour	Black
Pollen colour	Yellow
100 seed weight (g)	3.9
Volume weight (g/100 ml)	44.0
Average oil content (%)	41.32
Leaf blistering	Medium
Leaf petiole pigmentation	Absent

Associated characters and cultivation practices: This genetic stock is medium maturing and is recommended for utilization in heterosis breeding programme for development of high oil yielding hybrids.

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66. AAC-1 (IC00625184; INGR21066), a China aster (*Callistephus chinensis*) Germplasm with Resistance to Alternaria Leaf Spot Disease. High Yielding. Branching Habit and Late Flowering Type.

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China aster is one of the most important flower crops grown in Karnataka after rose, chrysanthemum and marigold and which is grown commercially for cut flowers, loose flowers and also in garden display and decoration purpose. The pure line AAC-1 is an open pollinated seedling selection from germplasm maintained at Department of Floriculture and Landscape Architecture (FLA), KRC College of Horticulture, Arabhavi. The unique characteristic of this variety is resistant to *Alternaria* leaf spot. Which was screened under both natural and artificial disease conditions.

Morpho-agronomic characteristics: Flowers of this pure line (AAC-1) are brick red in colour, flower diameter is 5.5-6.0 cm and flower weight 4.50 g, plant height is 55 cm, stalk length of 30 cm and takes about 138 days to flower from seed sowing. The plants produce about 50 flowers/plant.

Associated characters and cultivated practices: In Karnataka, it is mainly grown in districts viz., around Bangalore, Belgaum, Chitradurga, Tumakur and Davanagere. It can be grown in all seasons of the year, for quality flower production winter and rainy seasons are best. For seed production winter season is best.

China aster can be planted in ridge and furrow system. The spacing of 30 x 30 cm and fertilizer dose of 90:120:60 kg NPK/ha is recommended (as per UHS, Bagalkot package of practice) for its cultivation. The identified pure line AAC-1 has spreading growth habit and more biomass production. This pure line responds good at spacing of 45 x 45 cm and fertilizer dose of 112.5: 150: 75 kg NPK/ha for higher flower production and also a micronutrient combination of ZnSO₄

Morpho-agronomic description of China aster pure line AAC-1

Characters	Pure line AAC-1
Plant Height (cm)	56.09
Number of branches	16.77
Number of leaves	235.83
Leaf area/ plant (dm ²)	9858.67
Stem girth (cm)	1.82
Plant spread E-W(cm)	42.64
Plant spread N-S(cm)	37.65
Days taken for bud initiation	48.25
Days taken for first flowering	39.52
Duration of flowering (days)	37.73
1000 Seed weight (g)	2.14
Seed yield (g)/plant	5.54
Seed yield/ha (kg)	615.86
Number of flowers/plants	52.33
Individual flower weight/plant (g)	4.53
Flower yield/plant (g)	173.19
Flower yield per ha (t)	17.58
Flower diameter (cm)	5.07
Flower stalk length (cm)	24.18
Vase life (days)	7.20
Total Chlorophyll (mg/g)	1.27
Disease incidence (%) – <i>Alternaria</i> leaf spot	9.93

@ 0.4% + FeSO₄ @ 0.4%+ Boron @ 0.1% as tested at the department of FLA, KRC College of Horticulture, Arabhavi.

Before planting FYM at 10 t/ha along with half dose of above recommended fertilizers are applied. After planting 20-30 days earthing up is done to support the plants. At the time of earthing up remaining half dose of P and K and 25% of N are applied, remaining 25% N is topdressed after 60 days of planting.

The pest and disease management practices are followed as per UHS, Bagalkot package of practices.

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Web site: www.uhsbagalkot.edu.in

67. SBI/2020/GU 07-2276/266 (IC0636676; INGR21067), a Sugarcane (*Saccharum* sp.) Germplasm High cane yield (89.66 t/ha) under drought condition. Lowest reduction for single cane weight under drought. High Nitrogen (77.92 kg of dry biomass/kg of nitrogen). Use Efficiency with *Erianthus* Base

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Sugarcane is an important C4 crop cultivated in the tropics and subtropics for production of both sugar and bioenergy. Among abiotic stresses, drought causes severe yield losses worldwide in sugarcane. The present day cultivars are interspecific derivatives of *Saccharum officinarum* and *S. spontaneum* and the wild species *Saccharum spontaneum* has been the donor for many important traits including drought tolerance in sugarcane. In recent years, *Erianthus* spp. has been identified as a valuable source for important agronomic traits such as early vigour, biomass production, and ratoonnability, tolerance to biotic and abiotic stresses. The clone GU 07-2276 was developed by crossing two wild species viz., *Saccharum robustum* 'PIR 00-1188' and *Erianthus arundinaceus* 'IK 76-91' and backcrossing the resultant F1 'GU 04 (50) RE-9' with CoH 70 during 2007 at ICAR-Sugarcane Breeding Institute, Coimbatore. GU 07-2276 has tall, erect, light purple, cylindrical cane with slight zig zag alignment, green leaves with green leaf sheath, medium oval bud without bud groove.

Twenty-seven interspecific hybrids (ISH) and intergeneric hybrids (IGH) with diverse genetic base were evaluated for their drought tolerant potential at four sugarcane research stations under AICRP (Sugarcane) viz., Karnal (Haryana), Faridkot (Punjab), Anakapalle (Andhra Pradesh), Padegaon (Maharashtra) during 2016-17 to identify climate resilient

ISH and IGH genetic stocks for further use in sugarcane improvement programmes. The stress was initiated by withholding irrigation at formative phase ie 60DAP till 150DAP. The results showed that, for cane yield, the entry GU 07-2276 recorded the lowest reduction of -8.71% with a mean cane yield of 89.66 t/ha under drought. The entry also maintained higher relative water content and had shown only -2.27% reduction under drought compared to control. It also recorded the least reduction (-10.80%) for single cane weight compared to other entries under drought.

In another set of trials conducted during 2014-16, 32 pre-breeding genetic stocks with diverse genetic base were evaluated for Agronomic Nitrogen use efficiency (Good *et al.* 2004). The results showed that GU 07-2276 recorded higher dry biomass under N₀ (46.06 t/ha) and N₁₀₀ (57.67 t/ha) condition and also recorded the higher AgNUE of 41.40 with *Erianthus arundinaceus* base. Based on the results, the genetic stock GU 07-2276 has been identified as potential donor for drought tolerance combined with nitrogen use efficiency.

Reference

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68. Co 13003 (IC0638608; INGR21068), a Sugarcane (*Saccharum* sp.) Germplasm High fibre (15.05%) in Cane Combining High Sucrose (19.77%) Content of Commercial Level.

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Sugarcane is one of the important C4 crops, cultivated in more than 100 countries and contributing for more than 80% global sugar production. Though sugar is the main product, sugarcane is emerging as an energy crop in several countries including India, to meet the needs of clean energy and electricity. Fibre content in the canes and sucrose content in the juice are the major contributors for the primary energy content of sugarcane. These two characters are hard to combine (Huang, *et al.* 2017) and many wide hybrids and interspecific hybrids record high fibre and very low sucrose content. A clone combining these two characters is a potential source to meet energy demands. Co 13003 is one such rare recombinant combining high fibre per cent and juice sucrose content. Co 13003 is an early maturing clone selected from the cross involving two proven parents Co 86011 x CoT 8201. This clone was identified at ICAR-Sugarcane Breeding Institute, Coimbatore in the Pre zonal varietal trials conducted during 2012-13. The clone was selected as an early maturing clone with high early sucrose content of 18.07% at 240 days and 21.41% at 300 days at Coimbatore. The clone also recorded a cane yield of 145 t/ha with a sugar yield of 21.83 t/ha and was superior to the standards. Based on its superior performance, Co 13003 was promoted to AICRP (Sugarcane) and it was tested in 18 locations in 34 trials over two years. The trials were conducted with sixteen entries and three standards (Co 86032, CoC 671 & CoSnk 05103) in 14 centers under AVT IP during 2018-19 and AVT IIP during 2019-20.

In AVT I plant, the clone Co 13003 recorded the highest mean fibre of 15.15% compared to 15.07% in the best

standard, 13.51% in the standard and popular variety Co 86032 and 14.33% in the third standard CoC 6711. High fibre content of this clone was recorded at Kolhapur, Mandya and Padegaon. Its fibre in AVT Ratoon crop was 14.77%. The pooled analysis of two plant and ratoon also showed the superiority of Co 13003 for fibre % (15.00) compared to the checks Co 86032 (13.22%), CoC 671 (13.87%), CoSnk 05103 (14.93%). Co 13003 recorded 19.17% sucrose in AVT I Plant, 20.25 % in AVT II Plant and 19.98% in AVT ratoon trial. In a pooled analysis of two plant and ratoon data, it showed a mean sucrose of 19.77% and emerged as the best clone combining high fibre and high sucrose content.

Co 13003 has erect, light purple, heavily waxed, thick, erect canes, narrow purple growth ring, small bud, purple leaf sheath base with few hard spines, closed canopy with pointed to tip droopy leaves. It is sparse flowering late during the flowering season at Coimbatore. The clone combined high fibre of 15.05% and sucrose of 19.77% and recorded 14.51 t/ha sugar yield and 103.09 t/ha cane yield in AVT trials in the zone. Such a combination of high fibre with sugar yield component traits is very rare and beneficial when sugarcane is being looked upon as a food and energy crop yielding sugar, ethanol and electricity.

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69. AHCM-22-1 (IC-0621779) (IC00621779; INGR21069), a Lasora (*Cordia myxa*) Germplasm Resistance to Tingid Bug *Dictyla cheriani* Drake.

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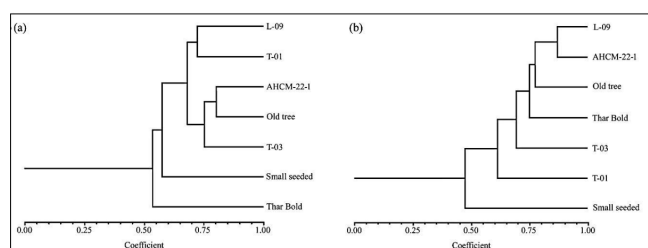
Lasora or gunda (*Cordia myxa*) is an important and underexploited perennial fruit plant of dry land. A wide range of genetic variability for horticultural attribute such as fruit size, shape and yield attributes is existed in seedling population of available in arid and semi arid region of Rajasthan. The plant is widely promoted as horticultural crop in particular to immature tender fruit for vegetable and pickle industries. The tingid bug, *Dictyla cheriani* is a major pest of lasora in India and its outbreaks cause substantial crop losses to growers. The lace bugs sucked the sap from newly emerging leaves and young branches, which led to the leaves turned yellow and suppression of growth of the tree through drying of leaves and young branches. The maximum incidence of tingid bug is usually observed in October in two different phenotypic characters (51.67% on bold & 76.67% on small seeded plants) and minimum

was in January (11.67% on bold & 21.67% on small seeded plants). The number of this lace bug ranged between (0.5 to 8.8 on bold & 4.5 to 25.97 on small seeded plants) nymphs and adults per leaves (Haldhar and Singh, 2014). A good amount of lasora germplasm is collected and 22 are conserved in field repository at ICAR-CIAH in May–July 2001. During the year, 2011 to 2013, the maintained lasora was evaluated for agro morphological traits, fruit yield and insect-pests incidence. Based on seasonal and over the year study, the germplasm AHCM-22-1 exhibited resistance towards to tingid bug. It is also high yielding and elite for fruit quality components. Therefore, its ripen fruits of AHCM-22-1 was collected, seedling progeny generated and plated in Entomology Block for further study.

The significant differences were found in percentage leaf infestation and bug density per leaf among the tested

Table 1: Salient characteristics of AHCM-22-1 (IC-0621779)

Genotypes	Bug/ leaf			Bug infestation (%)			Resistance category
	2014-15	2015-16	Pooled	2014-15	2015-16	Pooled	
AHCM-22-1	3.96	4.40	4.18	12.01	12.43	12.22	R
Germplasm	Flavinoid content ^{***} (mg/g)	Tannins content ^{**} (mg/g)		Phenols Content ^{***} (mg/g)	Total alkaloids ^{**} (%)	Free AminoAcid (mg/g)	
AHCM-22-1	5.43	22.38		18.44	0.43	0.87	
Germplasm	Leaf length (cm)	Leaf width (cm)		Leaf size	Leaf roughness	Leaf hairyness	
AHCM-22-1	14.62	12.88		Large	High rough	High hairy	

**Figure 1:** Phylogenetic tree construction using analysis of ScoT (a) and CBDP (b) markers

germplasm. Based on Kaiser Normalization method, the germplasm AHCM-22-1 was found resistant. The minimum bug density was observed in AHCM-22-1 (4.27 bugs/ leaf) followed by AHCM-25 (4.50 bugs/ leaf) and AHCM-34 (5.53 bugs/ leaf). The per cent leaf infestation was the highest in AHCM-32 (68.51 %) and the lowest in AHCM-22-1 (12.26 %) followed by AHCM-25 (14.25 %). The significant negative correlation was observed in AHCM-22-1 with percent bug infestation and total phenol, flavinoid and tannin content. The resistant germplasm AHCM-22-1 having leaf size (very large leaf), leaf roughness (high rough) and leaf hairyness (high hairy) and being high in resistant and low in susceptible germplasm accession. The germplasm AHCM-22-1 is

tolerating drought, high temperature and fruit yield of 27.50 to 39.80 Kg per plant after 4 to 5 years of commercial harvesting. Therefore, identified tinged bug resistant line namely AHCM-22-1 was molecularly characterized using random functional molecular markers. To complete this study, 32 ScoT and 17 CBDP markers were analyzed for their polymorphism and informativeness in the 7 germplasm including AHCM-22-1 and 6 germplasm. Six germplasm including a *C. myxa* variety, Thar Bold were used to compare the molecular pattern with AHCM-22-1 line. As the result, it clearly demonstrated that the AMCM-22-1 line was distinct from other related and compared germplasm and makes a separate group in phylogenetic analysis.

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70. AMMOL (IC0637592; INGR21070), an Apple (*Malus domestica*) Germplasm Better Fruit Size (higher (155g)). Early Maturity (114–117 days). Better Fruit Quality.

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Hybridization programme in indigenous apple cultivar “Ambri” was initiated to enhance the fruit quality parameters viz a viz early maturity. Hybridization of apple cultivar “Ambri” and early maturing Cultivar: Mollies Delicious” was done to obtain the desirable hybrid with better quality early maturing fruits.

Morpho-agronomic characteristics: Ammol is hybrid between Ambri and Mollies Delicious. Harvesting date is three weeks later than Mollies Delicious and forty days prior to Ambri i.e. in 4th week of August. Tree has moderate vigour with semi-spreading nature of growth habit suitable for high density plantation on clonal rootstocks.

Associated characters and cultivation practices: Fruit size is higher (155 g) than parents Ambri (140 g) and Red Delicious (145 g) with moderate acidity (0.23%). Anti-oxidative and free radical scavenging potential is significantly higher. DDPH

activity of hybrid is 28.1 μM AAE/g FW against Ambri (19.6 μM AAE/g FW) and Molles Delicious (26.0 μM AAE/g FW) and FRAP activity is 220 μM $\text{FeSO}_4/100\text{g}$ FW in comparison to Ambri (179.4 μM $\text{FeSO}_4/100\text{g}$ FW) and Molles Delicious (102.1 μM $\text{FeSO}_4/100\text{g}$ FW)

71. PRIDE (IC00638609; INGR21071), an Apple (*Malus domestica*) Hybrid with Scab Resistance . Fruit Quality (High TSS (18oB) and Higher firmness).

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Hybridization programme for development of scab resistant apple cultivars was initiated by introgression of scab resistant traits from apple cultivar "Prima" into commercial apple cultivar "Red Delicious". Hybridization of apple cultivar "Red Delicious" and scab resistant cultivar "Prima" was done to obtain the desirable hybrid with scab resistance trait.

Morpho-agronomic characteristics: PRIDE is hybrid between Prima and Red Delicious. Tree is having medium vigour with spreading branches. Stem vigour is medium with brown colour and twigs are medium to long with dark brown shade.

Associated characters and cultivation practices: Fruit is juicy, globose, medium size, has medium stalk cavity with small lobes, large overcolour, strong bloom of skin and graziness, fleshed stripped and mottled pattern of

overcolour, rust free, flesh creamish in colour, high TSS with good firmness. Fruit size is moderate (130 g) with respect to parents Prima (120 g) and Red Delicious (145 g) with moderate acidity (0.30%). Fruits have higher TSS (18°B) than Prima (12.5°B) and Red Delicious (14.3°B). Firmness of fruits is also higher (65 RI) than Prima (60 RI) and Ambri (64.2 RI) which contributes is higher shelf life. Fruits have higher phytoanticipins having 160 mgGAE/100g FW phenols, 130 mg QE/100g FW flavanoids and 1.79 mg QE/100g FW flavanols. Anti-oxidative and free radical scavenging potential is moderate. DDPH activity of hybrid is 23.51 μM AAE/g FW against Prima (20 μM AAE/g FW) and Red Delicious (28.0 μM AAE/g FW) and FRAP activity is 158 μM $\text{FeSO}_4/100\text{g}$ FW in comparison to Prima (183 μM $\text{FeSO}_4/100\text{g}$ FW) and Red Delicious (106.4 μM $\text{FeSO}_4/100\text{g}$ FW).

Specific trait: PRIDE is Scab Resistant as Prima

72. Arka Supreme (CHES-PA-III-1) (IC00612469; INGR21072), an Avocado (*Persea americana*) Germplasm High yield (about 370-400 kg/plant with average fruit weight of 367-428 g). Improved fruit quality. Regular bearing behaviour.

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Avocado is the most nutritive among fruits and is regarded as the most important contribution of the New World to human diet. Avocado is a nutrient dense and high oil content fruit. Avocados have the highest energy value (245 cal/100 g) of any fruit besides being a reservoir of several vitamins and minerals. The fruit has the highest fibre content of any fruit and is a source of antioxidants. The avocado (*Persea americana* Mill.) is also known as alligator pear and butter pear/butter fruit. It originated in Mexico and Central America, possibly from more than one wild species (Dreher

and Davenport, 2013). The pulp is rich in proteins (up to 4%) and fat (up to 30%), but low in carbohydrates. Avocado production of the world was 6.4 million metric tons in 2018 (Anonymous, 2019). Mexico is the largest producer and exporter of Avocado in the world followed by Chile, Indonesia, United States, Dominican Republic, Colombia, Brazil, Peru etc., while its production in Asia is limited. In India, it is cultivated on limited scale and in a scattered way in Tamil Nadu, Kerala, Maharashtra, and Karnataka in the south-central India and in the eastern Himalayan state

Table 1: Fruit yield and quality characteristics of avocado selections (mean of three years)

Parameters	CHES-PA-III-1 (Arka Supreme)	CHES-PA-XIII-1	TKD-1
Fruit yield (kg/tree)	254.7	209.8	198.5
Fruit weight (g)	389.4	472.4	289.3
No. of fruit/ tree	956.1	356.8	411.5
TSS (°Brix)	8.1	7.3	8.3
Stone wt. (g)	170.8	192.7	122.3
Pulp %	56.1	51.8	62.6

Table 2: Biochemical parameters of avocado selections

Biochemical parameters	CHES-PA-III-1 (Arka Supreme)	CHES-PA-XIII-1	TKD-1
Moisture (%)	82.3	79.4	81.7
Carbohydrates (mg/g defatted dry wt.)	40.5	36.4	42.7
Protein (mg/g defatted dry wt.)	13.2	11.2	14.6
Fat (%)	20.0	16.8	23.8
Fibre (%)	0.45	0.48	0.41

of Sikkim (Gosh, 2000). During recent past its cultivation is gaining momentum as cash crop, owing to its high nutritional value, high market potential and less resource demanding nature of crop.

In India, avocado is not a commercial fruit crop. It was introduced from Sri Lanka in the early part of the 20th century. Avocados are grown scattered in southern tropical states like Tamil Nadu, Kerala, Karnataka (Coorg), and Maharashtra. Also popular in the northeastern Himalayan state of Sikkim on hill slopes at elevations if 800- 1600 meters (Gosh, 2000). The climate zone of avocados is from true tropical to warmer parts of the temperate zone. In Kodagu, avocado is grown as one of the mixed crops in coffee-based cropping system. Almost each house is maintaining few plants of avocado and lots of variability of Avocado is available in Coorg and adjoining areas (Tripathi *et al.*, 2014).

Varietal improvement in Avocado has so far been limited to selection of regular bearing and high yielding genotypes with improved fruit quality. The present study considering the emerging importance of the crop was therefore carried out with the objectives of identifying regular bearing and high yielding genotypes with higher nutritive value.

Avocado improvement work was started at ICAR – IIHR-Central Horticultural Experiment Station, Chettalli during 2000 with an objective to identify high yielding type with regular in bearing type. Extensive surveys were made in Southern tropical states like Tamil Nadu, Kerala, Karnataka (Coorg), and Northeastern Himalayan state of Sikkim for the identification of high yielding accession with regularity in bearing, as irregularity in bearing is major hindrance in avocado cultivation. The criteria adopted for selection of superior genotypes: regular bearer, higher yield, medium size fruits, soft and buttery pulp and higher fat percent.

Passport data was recorded and in situ evaluation was carried out.

During the survey, out of 67 accessions, 17 accessions were evaluated for basic horticultural traits. The process resulted in identification of two superior accessions conforming to the criteria of selection outlined as above. Based on this, following selections made are detailed out and described here below:

CHESPA-III-1 (Arka Supreme)

It is a regular bearing and high yielding seedling selection from local collection with spreading type growth habit. A fully grown tree (16 year old) gives the fruit yield about 200-250 kg/plant with average fruit weight of 367-428 g. The fruits are oblong with 7.80 Brix TSS. The flowering behavior of this variety falls under Type "A" category. Total fat content is 20.0 %.

The different fruit parameters that were considered for evaluation of the accessions are listed in Table 1.

From above it may be observed that CHES-PA-III-1 (Arka Supreme) was found to be regular bearing and high yielding seedling selection from local collection with spreading type growth habit. The fruits are oblong, light green, pale yellow pulp with prominent lenticels on shoulders (Table 1 &2).

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73. SM/11-120 (IC0637593; INGR21073), a Potato (*Solanum tuberosum*) Germplasm Highly Resistant to Potato Cyst Nematode (*Globodera pallida* and *G. rostochiensis*). Highly Resistant to Late Blight (*Phytophthora infestans*) and Non Preference for White Fly. Performing Well Under Long Day Conditions

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Potato (*Solanum tuberosum* L.) is the 3rd important global food crop. It produces more food per unit of land in comparison to major crops, and its demand is growing continuously. In India too, potato has become a staple crop of masses and is grown in wide agro-ecologies (Pradel *et al.*, 2019; Sood *et al.*, 2020). Potato cultivation in diverse areas attract many biotic stresses which affect potato production in India. Major among them are late blight, viruses and potato cyst nematode (PCN) (Bhardwaj *et al.*, 2019). Development of new resistant varieties with higher productivity and other desirable traits is a continuous process. Host resistance, is cost effective, eco-friendly and easy to deploy strategy, which requires identified and known sources for resistance. Due to major focus on late blight and viruses resistance, breeding efforts have resulted in identification and successful deployment of resistance in newly developed varieties. Recently, PCN has emerged as a major problem in Northern hills in India and till date there is no highly resistance source for combating the disease. Moreover, white fly infestation transmits the viruses in potato tubers and affects health and quality of tubers.

Hybrid, SM/11-120 is a promising clone highly resistant to both the species of potato cyst nematode (*Globodera rostochiensis* and *Globodera pallida*), resistant to late blight disease (*Phytophthora infestans*) and showed resistance/non-preference to white fly. The hybrid was developed through biparental crossing between CP 2379 and Kufri Himalini. Kufri Himalini is a medium maturity adapted variety with good tuber attributes and is moderately resistant to late blight. CP 2379 is an advanced exotic line resistant to late blight and PVY. The clone was selected from the segregating population for late blight resistance under controlled conditions through artificial inoculation in year 2012. The clone was subsequently multiplied for tuber increase and evaluated for field resistance to late blight, potato cyst nematode, white fly and agronomic superiority. The clone is highly resistant to PCN, resistant to late blight, white fly and exhibited high yield with medium to late maturity. It

produces round yellow pink tubers with medium deep eyes and light yellow flesh. The hybrid SM/11-120 did not observe even a single cyst of both the PCN species in the root balls during three years of evaluation under controlled conditions. The hybrid also showed least preference to white fly under choice assay. Besides, the hybrid is resistant to late blight and has shown agronomic superiority over best controls at Kufri. With these parameters, SM/11-120 is an exceptional hybrid/parental line suitable for PCN, late blight and white fly resistance breeding in developing new potato varieties.

In advanced generation yield trials, SM/11-120 consistently out yielded all the controls for total and marketable tuber yields at 120 days crop duration at Kufri. The hybrid out yielded the control variety, Kufri Girdhari by margin of 71.6%, Kufri Himalini by 68.75% and Kufri Jyoti by 238.5% for total tuber yield over last 4 years. During three years of evaluation under controlled conditions the hybrid observed highly resistant reaction to both *G. rostochiensis* and *G. pallida* and not even a single cyst of both the species of PCN was observed in root balls of the hybrid, while all other hybrids and control varieties were susceptible and highly susceptible to both the species (Table 1; Fig. 1). The hybrid possesses consistently high level of resistance against late blight over the years which was at par to best control Kufri Girdhari. The mean AUDPC for four years in SM/11-120 was 19.8 whereas best control variety, Kufri Girdhari recorded mean AUPDC of 18.4, which much lower than other control varieties viz., K. Jyoti (1654.2) and K. Himalini (789.7) (Table 2). The hybrid leaves also showed least preference to white fly under choice assay in comparison to control varieties and other hybrids.

Molecular markers data also supported its resistance to PCN as the hybrid showed resistant band for *H1* gene markers TG689 and 57R specific to *G. rostochiensis* and *Gpa2QTL* markers *Gpa2-1* and *Gpa2-2* specific to *G. pallida*. The hybrid also showed resistant band for late blight (*cLET5E4_R3*, *R1-CosA*) and PVY (*YES3A* and *Ry_{adg}*) resistant genes.

Table 1: PCN resistance of advanced hybrid, SM/11-120 under controlled conditions

Hybrids/ controls	<i>Globodera rostochiensis</i>				<i>Globodera pallida</i>			
	2018	2019	2020 (Kufri)	2020 (Shimla)	2018	2019	2020 (Kufri)	2020 (Shimla)
SM/11-120	HR	HR	HR	HR	HR	HR	HR	HR
K. Girdhari	S	HS	HS	HS	S	HS	HS	HS
K. Himalini	S	HS	S	HS	S	HS	S	HS
K. Jyoti	S	HS	HS	HS	S	HS	HS	HS

Table 2: Late blight resistance of advanced hybrid, SM/11-120 at Kufri

Hybrids/ controls	AUDPC				Average
	2017	2018	2019	2020	
SM/11-120	0	0	77.3	2	19.825
K. Girdhari	14.5	23.5	19.7	16	18.425
K. Himalini	543.3	265.63	1180	1170	789.7325
K. Jyoti	1609	994.38	2020	1993.33	1654.178

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74. MSH/14-129 (IC0637594; INGR21074), a Interspecific Somatic Hybrid of Potato (*Solanum tuberosum*) derived from [cv. Kufri Gaurav × somatic hybrid 'P2' (*S. tuberosum* + *S. pinnatisectum*)] with Wider Genetic Base. High Yield Combined with Moderate Late Blight Resistance.

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Inter-specific potato hybrid MSH/14-129 is a back-cross progeny of advanced stage clonal selection at seventh generation (BC₁-C₇). MSH/14-129 was developed by crossing between Kufri Gaurav and somatic hybrid 'P2'. The clone 'P2' an inter-specific somatic hybrid developed by protoplast fusion between *Solanum tuberosum* dihaploid 'C-13' and diploid wild potato species *S. pinnatisectum*. MSH/14-129 possesses wider genetic base into cultivated (*S. tuberosum*) from wild species (*S. pinnatisectum*) with high tuber yield (avg. 42.84 tonnes/hectare) and moderate resistance to late blight disease under the natural (hot-spot) field conditions at Kufri, Shimla. This shows successful utilization of somatic hybrid 'P2' in developing a promising advanced stage hybrid (MSH/14-129) for high yield combined with late blight resistance to be used as a parental line in potato breeding in future. Additionally, molecular profiling using SSR markers (STU: 174, 179, 182, 190, 200 bp; and STIKA: 152, 198, 201, 235, 242, 245 bp) were revealed for genetic fidelity purpose. A few DUS descriptors of MSH/14-129 are: white-green sprout, semi-compact plant foliage, medium plant height, green stem colour, open leaf structure, large leaf length, broad leaf width, no anthocyanin colouration on flower bud, white flower corolla, small flower size, orange anther colour,

normal anther cone, normal pistil, longer stylar length, light yellow tuber colour, smooth skin, oval shape, shallow eye depth and light yellow tuber flesh colour. This inter-specific potato hybrid with diverse genetic background, high yield and moderate resistance to late blight disease under hot-spot conditions has potential to employ in potato breeding programmes to widen the genetic base of the cultivated gene pool.

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75. MCD24 (IC0637595; INGR21075), a Diploid Wild Potato (*Solanum microdontum*) Highly Resistant to Late Blight Disease.

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The elite genetic stock MCD24 was developed at ICAR-Central Potato Research Institute, Shimla by Clonal Selection method from several clones regenerated from true potato seed (TPS) of wild species (*Solanum microdontum*, Accession no. PI218224). MCD24 is an important genetic material for fusion parent in somatic hybridization and parental line in diploid breeding programme for introgression of very high resistance to late blight trait with wider genetic base into cultivated potato and also basic research studies on diploid potato breeding. Since potato is native to Lima, Peru (South America) and under the routine germplasm procurement programme of the institute. Our finding shows successful utilization of clonal selection method in development of elite genetic stock MCD24 for high resistance to late blight over four seasons under controlled conditions by challenge inoculation. DNA fingerprinting of MCD24 revealed SSR alleles (STU: 171, 178, 182, 184, 187, 190, 197 bp, and STIKA: 188, 192, 214, 218, 230, 234 bp) for genetic fidelity. A few DUS descriptors of MCD24 are: red-purple light sprout, compact plant foliage, small plant height, green stem colour, intermediate leaf structure, small leaf length, narrow leaf width, oval leaflet shape, white flower corolla, small flower corolla size, yellow anther colour, normal anther cone,

normal pistil, longer stylar length, late maturity, white cream tuber skin colour, smooth skin type, ovoid tuber shape, shallow eye depth and white tuber flesh colour. This diploid wild species MCD24 with diverse genetic background having very high resistance to late blight disease has potential to widen the gene pool of the cultivated potato.

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76. IC-629872 (IC0629872; INGR21076), a Betle Leaf (*Piper betle*) Germplasm with Dark Green Deep Concave Leaves with Wavy Margins and Low Eugenol (27.57%) Content.

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Betelvine (*Piper betle* L.) leaves is used directly for chewing as raw which also carries medicinal plant where its leaves are used for remedial purpose. It is perennial aromatic plant and belongs to family Piperaceae. Leaves bear property like antidiabetic, antiulcer, antitumor, cardiogenic, antioxidant etc. It is also rich in potassium, phosphorous, calcium, protein, fiber, starch, eugenol etc. Essential oil is extracted from the leaves had a fragrance and is used as an industrial raw material for manufacturing food additives, perfume, medicine etc. It is being commercial cultivated in West Bengal, Assam, Andhra Pradesh, Bihar, Tamil Nadu, Karnataka, Kerala,

Madhya Pradesh, Uttar Pradesh, Maharashtra, Odisha and small part of Chhattisgarh in India. Demand of this crop is increasing both in national and international market. At IGKV, Raipur (C.G) the strain IC629872 is selection from land races developed from single plant selection, purification and multiplication of landrace. IC629872 is a dark green leaf colour, deep concave shape leaves, wavy margin in leaves, high protein and low eugenol (27.57%) content. It was compared with Bidhan Paan-1 developed by BCKV, Kalyani and is commercially grown by the farmers.

Table 1: Marketable leaf yield in numbers per plant

Entry	Agroshed net		Poly tunnel	
	IC-629872	Bidhan Paan-1 (check)	IC-629872	Bidhan Paan-1 (check)
2017-18	75.92	81.92	73.04	77.28
2018-19	85.82	93.42	82.72	80.87
Mean	80.87	87.67	77.88	79.08

Table 2: Morphological description of betelvine strain IC-629872 in comparison with check variety Bidhan Paan-1

<i>Morphological descriptor</i>			
1.	Name of crop betelvine	IC-629872	Bidhan Paan-1 (Check)
2	Leaf color	Dark Green	Green
3	Internodes Color	Green	Green
4	Leaf Surface	Cariaceous	Cariaceous
5	Leaf Lamina Shape	Ovate Oblong	Ovate Oblong
6	Main Vein Let Number	7	7
7	Leaf Lamina Orientation Along Mid Rib	V Shaped	Flat
8	Leaf apex shape	Acute	Acute
9	Adventitious root production	Many	Many
10	Stem colour	Moderate Green	Moderate Green
11	Shelf life	17 days	17 days
<i>Quantitative Traits:</i>			
1	Leaf length (cm)	6.3	10.8
2	Petiole length(cm)	5.73	6.19
3	Leaf width (cm)	5.97	7.73
4	Leaf area (cm ²)	70.47	70.15
5	L/B ratio	1.75	1.30
6	Internodes length (cm)	8.10(more)	6.78(less)
7	Lamina length (cm)	10.41	10.05
8	Depth of sinus (cm)	1.13	1.08
9	Width of lobe (cm)	2.98	3.87
10	Ratio of depth of sinus & with of lobe	1.38	0.21
11	Leaf thickness (cm)	0.313	0.380
12	Leaf weight (g)	99.20	106.56
13	Specific leaf weight (mg/cm ²)	5.21	4.80
14	Leaf area index	6.52	6.05
15	R-value	0.92	1.23
16	Leaf yield (g)	85.82	98.72
<i>Qualitative traits:</i>			
1	N(g/100g)	3.58	3.48
2	P(mg/100g)	745	991
3	K(mg/100g)	30.7	39.5

4	Ca(mg/100g)	380	396
5	Protein(g/100g)	2.23	2.17
6	Fiber(g/100g)	2.61	2.02
7	Starch(g/100g)	4.39	4.36
8	Chlorophyll(mg/g)	2.3	2.23
9	Moisture content	3.23	5.11
10	Oil (ml/500g leaves)	0.5-0.8	0.5-0.8
11	Eugenol content %	27.57	49.31

Morph agronomic characterization: IC-629872 collection from selection has dark green leaf colour, V-shaped leaf lamina orientation along mid rib whereas green colour leaf and flat leaf lamina orientation along mid rib of the check. Leaf length is 6.3cm, petiole length is 5.73cm, leaf width is 5.97cm, eugenol content is 27.57% which is less than Bidhan Paan-1 (check) for all the traits. IC629872 was found high for specific leaf weight (5.21mg/cm²), leaf area index (6.52) and intermodal length (8.10cm), protein content (2.23g/100g) as compare to check.

MLT Data

The candidate strain (IC629872) was studied for its genetic diversity using molecular marker (ISSR and DAMD) which indicate that the strain is genetically diverse from check.

Associated characters and cultivation practices:

Betelvine is cultivated in Protected structure in C.G. Deep ploughing is done during early summer. After ploughing upper soil is left exposed for one to two month to reduce soil borne pathogens. During onset of monsoon two to three ploughing with harrow is done. After that 500kg vermicompost in 500sm area is mixed in soil. The raised bed 45cm above surface is prepared to facilitate drainage from the field.

Cuttings with one or two nodes along with attached leaves are used as a propagating material. Established cutting can also be used for planting. Before planting furrows are made. In the furrow ridges are constructed. In the ridges two rows of budded vine with mother leaf is planted at 30cm plant to plant spacing and row to row spacing 45cm. For 500 sq.m 4000 planting material is sufficient. Before planting cutting are dipped in 1% of Bavistin and dried for 10-15 minute and the basal region along with node are dipped in 0.5% rootex (GA-3) for high germination percentage. Dibbling method is used for planting. Whole is made with help of stick or khurpi so that without damaging internodes below the bud point is dipped in soil. Hole is completely packed with help of thumb and finger. After that for fast sprouting the plant is covered with straw. The field is to be irrigated twice a day with help of water cane /drip/ sprinkler for twenty days and after that straw is removed carefully.

Fertilizers for 500 sq.m area is 100kg (19:19:19) in 12 split/ year along with foliar spray of 2% in 15 days interval.75kg oil cakes in four split in three month interval and vermicompost

500kg. During summer irrigation is given almost every day in new plant and 4-5 days interval in old plants. In winter season irrigation is reduced to fortnight interval. During rainy no irrigation is required unless there is long dry spell. Chemical spray to control pest should be avoided. Neem kernel oil 5% concentration is sprayed in field if there is infestation of mealy bug or leaf eating caterpillar. Wettable sulfur 0.5-1% is applied if there is mites. *T. viridii* 5-7g/lit of water two times in a month as basal application along with foliar application 3g/15lit which control most of the diseases. Drenching with 1% bordex mixture at 60 days interval. During November and June lowering of vines is carried to

earthing the vine on soil surface before 30 cm to touches roof. Therefore, each vine is supported with help of nylon rope of 1mm thickness. Auxiliary branches are removed from main vine regularly. Harvesting of leaves starts after 7-8 month from the time of planting. In rainy season frequent harvesting is done but leaves are picked throughout the year when the leaves get mature from each plant 87 marketable leaves can be harvested.

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77. HV 2006-6 (IC00574228; INGR21077), a High Yielding Sun-Cured Chewing Tobacco (*Nicotiana tabacum*) Germplasm with Caterpillar Resistance.

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Chewing tobacco (*Nicotiana tabacum*) is one of the important commercial crops grown in Tamilnadu mainly for chewing purpose. In the chewing tobacco producing zone of Tamil Nadu tobacco, caterpillar (*Spodoptera litura*) is a major insect pest that causes serious economic loss to the growers. None of the varieties presently in cultivation in the zone is resistant to this pest. Hence, a caterpillar resistant cultivars line, HV.2000-6 was developed through through back cross breeding using Abirami, a sun cured chewing tobacco line as recurrent parent and DWFC as donor for caterpillar (*Spodoptera litura*) resistance (ICAR-CTRI 2006 & 2007). This line is morphologically akin to the caterpillar susceptible variety Abirami. The line shows its superiority in terms of resistance to tobacco caterpillar both under natural and artificial conditions (Table 1). It has an yield potential of about 3950 kg/ha as observed in various AINPT trials. Hence registered as a high yielding caterpillar resistant sun-cured chewing tobacco line.

Morpho-agronomic characteristics: HV.2000-6 plants grows to a height of 1.4 m to 1.5 m under un-topped condition and 0.4 m to 0.6 m when topped. Plant are open in habit, leaves thick, shiny dark green, 75-80 cm long and 45-48 cm wide with good auricle development, prominent mid-rib acute tip and heavy puckering. Panicle is semi-open and branched. The days taken for seeding to transplanting is 45-50 days, transplanting-flowering 60-65 Days and seed to seed 155 – 165 days. Cured leaves have dark brown color, heavy bodied, elastic with whitish incrustations,

Table 1: Reaction to Leaf eating caterpillar

Condition	Year	HV.2000-6	Abirami
Artificial	2000-01	100% resistant to first instars larvae.	Susceptible
	2001-02	100% resistant to first instars larvae.	Susceptible
	2002-03	100% resistant to first instars larvae.	Susceptible
Natural (% plants affected)	2003-04	-	48.0
	2004-05	1.2	64.8
	2005-06	1.6	56.8
	Mean	1.4	56.5

sweet aroma and medium strength. HV 2000-6 recorded higher mean scores for various leaf quality characteristics by Trader's (62.2 out of 80) and Consumer's (59.6 out of 80) than control Abirami (57.5; 56.4, respectively).

Associated characters and cultivation practices: The recommended cultural practices for chewing tobacco can be adopted for rising HV.2000-6. The entry is susceptible to other pests and diseases and its reaction is comparable to control, Abirami.

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78. NLCR 6-10 (IC0634528; INGR21078), a FCV Tobacco (*Nicotiana tabacum*) somaclone with High Yielding Cured Leaf Having More Number of Longer and Broader Curable Leaves. Suitable for Irrigated Alfisols.

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Flue-cured Virginia (FCV) tobacco is the major exportable tobacco type grown in India. FCV tobacco grown in the irrigated alfisols of the Northern Light Soils (NLS) covering West Godavari district of Andhra Pradesh and Khammam district of Telangana is known for its premium leaf quality and is exported to several countries. In order to increase the yield level and export competitiveness of the tobacco of the area, NLCR 6-10, a high leaf yielding somaclone was developed from Kanchan explants at ICAR- Central Tobacco Research

Institute Research Station, Jeelugumilli (ICAR-CTRI 2011, 2012, 2013, 2018, 2019a & 2019b). The line NLCR 6-10 produces a total of 35 leaves with about 31 good bodied ones suitable for harvesting and curing under NLS (Table 1) compared to 29 curable leaves in control, Kanchan (2009-12). The leaves of NLCR 6-10 are longer (up to 88 cm) and broader (up to 44 cm) than Kanchan (up to 77 cm long and 35 cm broad). The entry, NLCR 6-10 is high yielding than Kanchan and has a leaf yield potential of more than 3300 kg/ha (Table 2).

Table 1: Leaf characters of NLCR-6-10 under replicated (2009-12) and on-farm (topped condition) trials

Year	Village	No. of curable leaves		Leaf length (cm)		Leaf width (cm)	
		NLCR-6-10	Kanchan	NLCR-6-10	Kanchan	NLCR-6-10	Kanchan
	Replicated trial						
2009-10	CTRI RS, Jeelugumilli	32 (36)	31 (33)	51	52	58	22
2010-11	CTRI RS, Jeelugumilli	26 (30)	26 (29)	66	60	58	18
2011-12	CTRI RS, Jeelugumilli	34 (38)	30 (33)	64	63	31	19
	Mean	31 (35)	29 (32)	60	58	31	20
	On- Farm trials						
2016-17	Bandapuram	-	-	83	74	36	33
	Mangaparthidevepeta	-	-	85	80	41	36
	Muppinavarigudem	-	-	81	73	36	33
Mean		-	-	83	76	38	34
2017-18	Bandapuram	-	-	84	77	40	36
	Mangaparthidevepeta	-	-	88	83	44	38
	Muppinavarigudem	-	-	83	75	37	34
Mean		-	-	85	78	40	36

*Figures in the parenthesis are total leaves

Table 2: Leaf yields of NLCR-6-10 under different trials (2009-2019)

Trial	Year	No. of trials	Green leaf			Cured leaf			Grade Index		
			NLCR-6-10	Kanchan	CD 5%	NLCR-6-10	Kanchan	CD 5%	NLCR-6-10	Kanchan	CD 5%
RYT	2009-10 to 2011-12	3	13847*(13)	12190	1026	2237*(16)	1927	177	1376*(31)	1053	118
IVT (JML)	2013-14	1	13153*(39)	9458	1711	2257*(34)	1689	312	1532*(46)	1049	212
AVT (JML)	2014-15 to 2015-16	2	22314*(41)	15815	1070	3309*(39)	2373	182	2179*(55)	1410	142
Bulk trial (JML)	2015-16 to 2018-19	4	19780 (48)	13371	-	3270 (47)	2227	-	1957 (66)	1177	-
On-farm trial	2016-17 to 2018-19	13	-	-	-	2568 (20)	2132	-	-	-	-

Note: RYT: Replicated trail; IVT: Initial Varietal Trial; JML: Jeelugumilli; AVT: Advanced Varietal Trial; * Significantly superior over Kanchan

Hence, recommended for registration as high cured leaf yielding FCV tobacco somaclone with more number of longer and broader leaves suitable for irrigated alfisols.

Morpho-agronomic characteristics: The leaves of NLCR 6-10 are dark green coloured, strongly puckered, broad elliptic and strongly recurved with strongly pointed tip. The cured leaf yield potential of FCJ-11 is 3981 kg/ha in AVT-1 (2014-15) at CTTRI RS, Jeelugumilli, which is the highest among the FCV cultivars. Cured leaf of NLCR 6-10 is bright lemon to orange in colour with good aroma.

Associated characters and cultivation practices: Under field condition, NLCR 6-10 recorded slightly lower incidence of pests (*Spodoptera*, budworm and aphids) and mostly similar reaction to major diseases compared to control, Kanchan. The chemical quality parameters of NLCR 6-10

were within the permissible range and are comparable with control, Kanchan.

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79. F6-2-2 (IC0638885; INGR21079), a Tobacco (*Nicotiana tabacum*) Germplasm High seed yielding chewing tobacco.

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Tobacco is a commercial crop grown in India, mainly, for its traditional uses viz., smoking, chewing, etc. However, it can also be exploited for the production of seed oil as tobacco plant is a prolific producer of tiny seed containing ~ 35% oil. This oil is mainly used in paints, varnishes, lubricants and soap industries. The studies conducted at ICAR-CTRI, Rajahmundry indicated that tobacco oil can be used for edible purpose as its nutritional quality is comparable to sunflower and ground nut oils and is free from tobacco related harmful substances. As a part of the drive to exploit tobacco for edible oil production, a high seed yielding chewing tobacco line, F6-2-2 was developed at ICAR-CTRI Research Station, Veda sandur (ICAR-CTRI, 2017, 2018, 2019a and 2019b). The lines was developed through pedigree method of breeding from a cross between A 145 (a high seed yielding bidi entry) and Bhagyalakshmi (a chewing tobacco entry). F6-2-2 found to record higher seed yield than control varieties during 2015-19 (Table 1). The seed yield of this line ranged from 750-1600 kg/ha with the mean yield of 1257 kg/ha compared to 599 kg/ha in control, Abirami and 766 kg/ha in Bhaghyalakshmi. F6-2-2 yielded more than double the seed yield of control, Abirami and 64 % higher than Bhagyalakshmi. Higher seed yield in F6-2-2 may be due to higher number of capsules per plant (303) than controls Bhagyalakshmi (90) and Abirami (137). Hence, this line is registered as high seed yielding chewing tobacco line.

Morpho-agronomic characteristics: The plants of F6-2-2 are conical in shape, open in habit, leaves are lanceolate

Table 1: Seed yield (kg/ha) of F6-2-2 and controls (2015-19)

Year	F6-2-2	Abirami	Bhagyalakshmi
2015-16	1512	800	1208
2016-17	1600	582	500
2017-18	750	460	634
2018-19	1167	552	723
Mean	1257	599	766

dark green in colour, 25 in number. During 2016-19, F6-2-2 yielded up to 3400 kg/ha cured leaf yield under topped condition. The inflorescence is medium, flowers are pink in colour, seed capsules are medium in shape and seeds are brown in colour.

Associated characters and cultivated practices: The recommended cultural practices for chewing tobacco can be adopted for rising F6-2-2 with a spacing of 75 cm x 60 cm. Its reaction to pests and diseases is comparable to controls, Bhagyalakshmi and Abirami.

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80. JS-117 (IC0625211; INGR21080), a Flue-Cured Virginia Tobacco (*Nicotiana tabacum*) Germplasm with Low smoke tar delivery

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Tar is the partially combusted particulate matter produced by the burning of tobacco in the act of smoking. The smoke tar content in Flue-cured Virginia (FCV) tobacco range from 22-38 mg/cigarette (33-55 mg/g dry tobacco). The tar content of tobacco is associated with some of the harmful effects of tobacco use. In view of this, ICAR- Central Tobacco Research Institute Research Station, Jeelugumilli bred a low tar delivering Flue-cured Virginia (FCV) line, JS-117. The lines developed was through pedigree method of breeding involving the high yielding and popular FCV tobacco cultivar, Kanchan and low tar di-haploid line, D-1 (Sarala *et al.*, 2012). It delivers 12% less tar per gram tobacco used or 11% less tar/cigarette compared to control, Kanchan (Table 1 & 2; ICAR-CTRI 2014 & 2015). Hence, JS-117 registered as low tar delivering FCV tobacco line.

Morpho-agronomic characteristics: JS-117 is a green cast line with semi erect habit and short internodes. It grows to a height of around 1.17 m. Plant shape resembles the ruling variety, Kanchan. The line gives more than 24 curable leaves. The leaves are long (65-72 cm) and slightly wider (24-34 cm) than Kanchan variety. Cured leaf is deep lemon to orange in colour, open grained, medium to thick bodied, semi-flavourful, rich in oil and fluffy. The line has a yield potential of about 2500 kg/ha. The physical and chemical characteristics of the variety, JS-117 are comparable with control, Kanchan and are in acceptable range.

In the station replicated trials (2003-2004 to 2005-2006), JS-117 recorded significantly higher leaf yields (18%) in cured leaf yield (2167 kg/ha) over Kanchan. In the IVT (2006-07) and AVT (2007-09) the line recorded a cured leaf yield of 2335 kg/ha and 2140 kg/ha (Pooled), respectively. In the station bulk plot trials conducted at ICAR-CTRI RS, Jeelugumilli (2009-10 to 2010-11), JS-117 recorded 15% superiority cured leaf yield over check, Kanchan. The line proved its superiority in on-farm trials (2011-12 and 2012-13) at four locations with an average of 21% increase in cured leaf yield (2565 kg/ha) over the control, Kanchan. In the agronomic trial (2012-14), a nitrogen dose of 130 kg N/ha and topping at 26 leaves were found to be optimum for realizing the yield potential of JS-117.

Associated characters and cultivated practices: JS-117 recorded lower natural incidence of Brown spot, *Fusarium* wilt, TMV and *Orobanche* compared to Kanchan. The reaction of JS-117 to other major pests and diseases is on par with Kanchan.

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 ICAR-CTRI (2015) Annual Report 2014-15. ICAR-Central Tobacco Research Institute, Rajahmundry, 16p

Table 1: Data on Smoke tar deliveries

Smoke tar (mg/cigarette)			Smoke tar (mg/g dry tobacco)		
Year	JS-117	Check variety, Kanchan	Year	JS-117	Check variety, Kanchan
2005-06	20.92(13)*	23.96	2005-06	41.89(3)*	42.97
2007-08	19.46 (12)	22.11	2007-08 ('X' position)	31.19 (20)	38.88
2008-09	19.27 (14)	22.40	2007-08 ('L' position)	40.72 10)	45.28
2011-12	21.20 (07)	22.88	2011-12	38.97 (9)	42.89
2012-13	22.08 (08)	24.10	2012-13	37.60 (18)	46.0
Mean	20.59 (11)	23.09	Mean	37.07 (12)	43.20

*Figures in the parenthesis are % reduction in tar delivered over control, Kanchan

81. Jayalakshmi (IC0637597; INGR21081), a Flue Cured Virginia Tobacco (*Nicotiana tabacum*) Germplasm with White flower and White (cream colour) seed

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Table 1: Flower and seed colour characteristics

Entry	Flower Colour				Seed Colour			
	2016-17	2017-18	2018-19	2019-2020	2016-17	2017-18	2018-19	2019-2020
Jayalakshmi	White	White	White	White	White	White	White	White
A-145 (C)	Pink	Pink	Pink	Pink	Brown	Brown	Brown	Brown
Siri (C)	Pink	Pink	Pink	Pink	Brown	Brown	Brown	Brown

Flue-cured Virginia (FCV) tobacco is the major exportable tobacco type grown in India. FCV tobacco grown in India. In general, FCV (*Nicotiana tabacum*) lines have pink flowers and produce brown seed. However, in the FCV tobacco germplasm, white flower and white seed (cream colour) producing entry, Jayalakshmi was identified. This is a line collected from the agricultural fields of East Godavari district, AP and is being maintained at the genebank of ICAR-CTRI, Rajahmundry. This line found to consistently give white flower and white seed (ICAR-CTRI, 2013, 2019a and 2019b).

This line being used in the development of a mapping population through crossing with A-145 an entry with pink flowers and brown seed in order to produce higher white seed yielding tobacco genotypes and to understand genes involved in seed and oil yield. Oil extraction from white colour seed results in clear seed oil and requires lesser processing compared to the oil extracted from brown seed. This assumes significance for exploring tobacco seed oil for edible purpose.

Morpho-agronomic characteristics: Jayalakshmi plants are conical in shape with semi-erect plant habit. Leaves are sessile, moderately acute, medium pointed, broad elliptic, medium to dark green, with medium blistering, medium wavy margins and undulations. Inflorescence loose and spherical in shape. Flowers are white in colour and produce white/cream colour seed.

Associated characters and cultivated Practices: Jayalakshmi (ws) recorded higher seed oil content (39%) than A-145 (37%) (ICAR-CTRI, 2013). The standard cultural practices recommended for FCV tobacco can be adopted in raising the entry.

References

- ICAR-CTRI (2013) ANNUAL REPORT 2012-13. ICAR-Central Tobacco Research Institute, Rajahmundry, 11 p
 ICAR-CTRI (2019a) ANNUAL REPORT 2018-19. ICAR-Central Tobacco Research Institute, Rajahmundry, 30 p
 ICAR-CTRI (2019b) ANNUAL REPORT 2019. ICAR-Central Tobacco Research Institute, Rajahmundry, 28 p

82. 1/135 (IC0637598; INGR21082), a Tobacco (*Nicotiana tabacum*) Germplasm with High Solanesol (3.43 %).

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Tobacco is mainly grown for its traditional uses viz. Smoking, chewing, snuff, Hookah etc. Tobacco can also be exploited for its alternative uses in the form of extraction of important native phytochemicals viz., solanesol, nicotine, organic acids, leaf protein etc. Solanesol is an important secondary metabolite available in tobacco having pharmaceutical importance in controlling cardiovascular diseases, aging etc. While carrying out research in the direction of alternative uses of tobacco at ICAR- Central Tobacco Research Institute, Rajahmundry, line 1/135 was developed from a cross between a high solanesol line, HDBRG and a low solanesol line, BY53 (Sarala *et al.*, 2018). When the population of pure lines developed from the above cross was tested for solanesol, the line, 1/135 recorded higher solanesol than

the high solanesol yielding line, HDBRG most of the years during 2014-17 and 2019-2020 as shown below. The line recorded a mean of 3.43 % with 73% mean increase over HDBRG during this period.

Associated characters and cultivation practices: The reaction of the line to major pests and diseases of tobacco under natural conditions is on par with HDBRG.

References

- Sarala K, K Prabhakara Rao, C Chandrasekhararao, D Damodar Reddy and K. Bagyalakshmi (2018) Phenotyping tobacco recombinant inbred lines for solanesol. *Intl J Chem Stud* **6**: 3643-3650.
 ICAR-CTRI (2019a) ANNUAL REPORT 2018-19. ICAR-Central Tobacco Research Institute, Rajahmundry, 30 p

Entry	Solanesol %				Mean
	2014-15	2015-16	2016-17	2019-2020	
1/135	4.9 (96)	3.6 (57)	2.8 (100)	2.4 (41)	3.43 (73)
HDBRG	2.5	2.3	1.4	1.7	2.00
By-53	1.4	1.7	0.6	0.70	1.23

*% improvement over high solanesol line, HDBRG

Morpho-agronomic characteristics		
Parameter	HDBRG	1/135
Plant Shape	Cylindrical	Conical
Plant Height (cm)	145	135
Plant Habit	Semi Erect	Semi Erect
Plant Internodal Length (cm)	3	4.5
Plant Number of Leaves	28	33
Leaf Type	Sessile	Sessile

Parameter	HDBRG	1/135
leaf angle of insertion	Moderately Acute	Moderately Acute
leaf length (cm)	53	33
Leaf Width (cm)	20	18
Leaf Midrib	Thin	Medium
Leaf veins thickness & Angle	Medium/ModeratelyAcute	Medium/ModeratelyAcute
Leaf Blade Shape	Narrow Elliptic	Broad Elliptical
Leaf Tip Shape	Strongly Pointed	Strongly Pointed
Leaf Blistering of Blade	Weak	Weak
Leaf Undulation of Margin	Weak	Weak
Leaf Development of Auricles	Medium	Medium
Leaf Colour of Blade	Medium Green	Green
Leaf Colour of Midrib	Green	Green
Time of 50% Flowering	70 Days	114 Days
Inflorescence Shape	Spherical	Spherical
Inflorescence compactness	Medium	Medium
Inflorescence Position Relative to Upper Leaves	Above	Above
Flower Colour	Light Pink	Pink
development of stamens	Full	Full
Fruit form	Intermediate	Ovate
Testa colour	Light Brown	Light Brown

83. V-4914 (IC0634529; INGR21083), a Flue-cured Virginia Tobacco (*Nicotiana tabacum*) Germplasm with High Yield and Resistance to Tobacco Mosaic Virus

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Tobacco Mosaic Virus (TMV) is one of the major diseases in tobacco causing yield and quality losses. In order to stabilise the yield levels of Flue-cured Virginia (FCV) tobacco grown in Southern Light Soil (SLS) areas of Prakasam and Nellore districts, Andhra Pradesh, an attempt made to develop TMV resistant lines. V-4914 (FCR 15) a high yielding (24500 kg/ha), TMV resistant light cast FCV tobacco cultivar suitable for moisture stress prone was developed by hybridization (*N. tabacum*, cv. Siri X *N. tabacum*, cv. VT 1158) followed

by pedigree selection after artificial inoculation in each generation (ICAR-CTRI, 2010, 2011, 2012, 2013 and 2019). The line V-4914 recorded resistance reaction to TMV under both artificial as well as natural conditions during 2015-19 (Table 1). Hence, registered as high yielding TMV resistant FCV tobacco cultivar.

Morpho-agronomic characteristics: V-4914 plant has semi erect habit, height 210 cm; Plant width at 5th leaf

Table 1: Reaction to TMV disease

S. No.	Year	V-4914	Control, Siri
Under artificial inoculation			
1	2015-16	R (0)	S (100)
2	2016-17	R (0)	S (100)
3	2017-18	R (0)	S(100)
4	2018-19	R (0)	S(100)
Natural Condition			
5	2016-17	R (0)	S (30)

Note: Figures in the parenthesis are % plants infected with TMV

position is about 80 cm as against 78 cm in Siri. Stem light green to cream coloured, internode short to medium (5 cm on an average). Leaf lamina is moderately long broad with good puckering and acute to acuminate tip. Leaf is light green cast in nature, sessile with medium auricle development. The average leaf length of 5th, 10th and 20th leaf are 56 cm, 58.4 cm and 29.5 cm respectively and the width is 29.5 cm, 30.4 cm and 14.5 cm respectively. The plant produces a total of 24-30 leaves with 22-27 economic leaves. During 2016-18, under 10 different AINPT trials, V-4914

(tested with the code FCR -15) recorded up to 48% increase in cured leaf over control, Siri at SLS areas of AP. The line found to have a yield potential of 2400 kg/ha.

Associated characters and cultivation practices: The recommended cultural practices for FCV tobacco grown under SLS conditions can be adopted for rising V-4914. Average weight of 100 cured leaves of V-4914 is about 510 grams against 470 g in Siri. The cured leaf is lemon yellow to orange in colour. Cured leaf is medium bodied, oily with good ripeness characteristics. The reaction to major pest and other diseases is similar to control, Siri.

References

- ICAR-CTRI (2010) ANNUAL REPORT 2009-10. ICAR-Central Tobacco Research Institute, Rajahmundry, 29p
 ICAR-CTRI (2011) ANNUAL REPORT 2010-11. ICAR-Central Tobacco Research Institute, Rajahmundry, 30p
 ICAR-CTRI (2012) ANNUAL REPORT 2011-12. ICAR-Central Tobacco Research Institute, Rajahmundry, 23p
 ICAR-CTRI (2013) ANNUAL REPORT 2012-13. ICAR-Central Tobacco Research Institute, Rajahmundry, 18-19p
 ICAR-CTRI (2019) ANNUAL REPORT 2019. ICAR-Central Tobacco Research Institute, Rajahmundry, 2p

84. BSR-1 (IC0634526; INGR21084), a Chewing Tobacco (*Nicotiana tabacum*) Germplasm with Resistance to Black Shank (*Phytophthora parasitica*)

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Black shank (*Phytophthora parasitica*) disease is a serious problem in chewing tobacco cultivated in the coastal belt of Tamil Nadu. An effort made at the CTRI RS, Veda sandur to incorporate black shank resistance into the existing pure line variety VR-2 without affecting yield, physical and chewing quality attributes. VR-2 was crossed with Black shank resistant donor, Beinhart 1000-1 and the resultant hybrid was back crossed to VR-2. Back crossing continued for four generations while selecting the plants resembling VR-2 and possessing black shank resistance. The stabilised entry, BSR-1 (mentioned as BC5S1 in CTRI Annual Reports) found to consistently perform well in the Research station as well as in the black shank sick fields in Veda ranyam areas of coastal chewing belt. In the endemic areas, BSR-1 recorded resistance reaction to black shank disease under both artificial and sick field conditions (Table 1) compared to controls, VR-2 and Kaviri. Hence, this line is registered as black shank resistant chewing tobacco line.

Morpho-agronomic characteristics: BSR-1 plants are open with dark-green, medium broad ovate leaves with prominent auricle and moderate puckering. Internodes are medium and

Table 1: Reaction of BSR-1 to black shank disease under inoculated and sick fields at Coastal chewing belt of Tamil Nadu (Hot spot area)

Condition	Year	BSR-1	VR-2	Kaviri
Artificial	2006-07	Resistant	Susceptible	Susceptible
	2007-08	Resistant	Susceptible	Susceptible
	2008-09	Resistant	Susceptible	Susceptible
Sick fields (% incidence)	2006-07	Nil	60.5	12.8
	2007-08	Nil	56.0	13.0
	2008-09	Nil	45.3	13.3
	Mean	Nil	50.6	13.0

stem girth moderate. BSR-1 recorded a mean yield of 2624 to 3687 kg/ha in different trials with 42% and 17% higher mean yields, covering eight trials, than controls, VR-1 and Kaviri, respectively.

Associated characters and cultivation practices: The recommended cultural practices for chewing tobacco can be adopted for rising BSR-1. Chewing quality characteristics are rated superior by traders and consumers than recurrent

parent, VR-2. BSR-1 is susceptible to other pests and diseases of tobacco and is comparable to controls, VR-2 and Kaviri. The farmers expressed satisfaction over raising BSR-1 as they could raise a healthy crop of tobacco without application of fungicide for black shank thus cutting down the cost on chemical fungicides thereby reducing production costs and increasing net returns besides being environment friendly.

References

- ICAR-CTRI (2008) ANNUAL REPORT 2007-08. ICAR-Central Tobacco Research Institute, Rajahmundry, 21p
 ICAR-CTRI (2009) ANNUAL REPORT 2008-09. ICAR-Central Tobacco Research Institute, Rajahmundry, 18-19 p
 ICAR-CTRI (2010) ANNUAL REPORT 2009-10. ICAR-Central Tobacco Research Institute, Rajahmundry, 30-31 p
 ICAR-CTRI (2015) ANNUAL REPORT 2014-15. ICAR-Central Tobacco Research Institute, Rajahmundry, 6 p

85. Cr-6017 (IC0636679; INGR21085), a Quality Tea (*Camellia sinensis*) Germplasm.

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The clone is selected from The Nilgiris, Tamilnadu. It is selected for its high-quality character for high grown orthodox tea. The genome of this plant has been sequenced under, "Tea genome re-sequencing project".

Morpho-agronomic characteristics: The clone is a diploid with medium sized leaves and dense crop shoots. Leaves are elliptic oblong in shape, young leaves are light green and are occasionally pigmented (pinkish tinge colour in young leaves).

Associated characters and cultivation practices: The clone is preferred for making high grown orthodox teas. It is a preferred scion clone for grafting on hardy root stock tea clones.

References

- Mohan M and VS Sharma (1981) Morphology and Systematics of some tea (*Camelliaspp.*). Proceedings of PLACROSYM IV: 391-400.
 Balasaravanan T, PK Pius and R Rajkumar (2002) Status of south

Indian tea germplasm. Newsletter UPASI Tea Research Foundation.12(2).

Biochemical characters

Biochemical parameters	Quantity % (mg/g) *
Polyphenol	21.16
Amino acid	1.05
Catechin	15.44
Caffeine	2.60
Anthocyanin	0.03
Carotenoids	0.20
Theaflavin	1.09
Thearubigin	6.10
High polymer substance	7.63
Total liquor colour	3.33

*Data presented here is the average value of 4 consecutive years reading.

86. UPASI-9 (IC0636680; INGR21086), a Drought Tolerant Tea (*Camellia sinensis*) Germplasm.

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The clone is selected from the old seedling tea population in The Nilgiris, Tamilnadu. The clone is selected for its high yield and drought tolerance character. The genome of this plant has been sequenced under, "Tea genome re-sequencing project".

Morpho-agronomic characteristics: The Clone is a diploid with dense crop shoots and flushing throughout the year. The leaves are elliptic-oblong or obovate-oblong, young leaves are dark green in colour and the flush is fairly larger in size, narrow dark green in colour with medium internodal distance.

Associated characters and cultivation practices: The clone is preferred by all the growers for its high yield of 5000 kg made / ha /year. It serves as an excellent root stock for most of the scion. The quantum increase in yield was found to be greater when it is used as a root stock for grafting.

References

- Venkataramani KS and VS Sharma (1975) Notes on the UPASI Tea clones approved by Tea Board and released for commercial planting. The hand book of tea culture. 1-3
 Mohan M and VS Sharma (1981) Morphology and Systematics of some tea (*Camellia spp.*). Proceedings of PLACROSYM IV: 391-400.

Morphological characters

Characters	Quantification
Leaf shape	Lanceolate
Total leaf length (cm)	14.3
Lamina length (cm)	13.8
Area of leaf in (mm ²)	4244
Lamina width (cm)	4.6
Ratio between Length and width of Lamina	0.33
Leaf venation	Sunken
Leaf margin	Serrate
Young shoot colour	Green
Individual shoot length (cm)	7.9
Individual shoot weight (g)	1.17
Internodal length (cm)	3.5
Internodal width (cm)	1.2
Stomatal conductance (mmol (H ₂ O) m ⁻² S ⁻¹)	151
Transpiration rate (mmol (H ₂ O) m ⁻² S ⁻¹)	2.07
Net photosynthetic rate ((CO ₂) m ⁻² S ⁻¹)	3.5

*Data presented here is the average value of 4 consecutive years reading.

87. UPASI-3 (IC0636681; INGR21087), a Triploid Tea (*Camellia sinensis*) Germplasm.

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The clone is selected from an old seedling population in The Nilgiris, Tamilnadu. It is selected for its excellent quality characters of black tea. The genome of this plants has been sequenced under, "Tea genome re-sequencing project".

Morpho-agronomic characteristics: The clone is a triploid with large leaves and succulent crop shoots. Leaves are large, often obovate or slightly obovate, elliptic in shape and bright green in colour. It is also a naturally occurring triploid which is rare in the tea gene pool that are primarily consists of diploid plants.

Associated characters and cultivation practices: This clone is one of the outstanding tea clones as it performs good at varying condition in varying elevation and climatic zone at different tea districts.

References

- Mohanani M and VS Sharma (1981) Morphology and Systematics of some tea (*Camellia spp.*). Proceedings of PLACROSYM IV: 391-400.
- Venkataramani KS and VS Sharma (1974) The Tea clone 'Sundaram', The hand book of tea culture. 1-3.

Biochemical characters

Biochemical parameters	Quantity % (mg/g) *
Polyphenol	23.22
Amino acid	1.87
Catechin	16.33
Caffeine	2.70
Anthocyanin	0.03
Carotenoids	0.22
Theaflavin	1.29
Thearubigin	8.13
High polymer substance	9.29
Total liquor colour	3.63
Chromosome number	3n=3(15) = 45

*Data presented here is the average value of 4 consecutive years reading.

88. ATK (IC0636684; INGR21088), a Tea (*Camellia sinensis*) Germplasm Drought Tolerance

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The clone is selected from old seedling tea population in the Nilgiris, Tamilnadu. The clone is selected for its drought tolerance character. The genome of these plants has been sequenced under, "Tea genome re-sequencing project".

Morpho-agronomic characteristics: This clone is a diploid with dense crop shoots, leaves are narrow elliptic-oblong in shape. Mature leaf is dark green in color whereas the young leaves contain a purple tinch.

Associated characters and cultivation practices: It is an easy rooting clone in the nursery and readily establishes in the field. The clone preferred for its drought tolerant character and is a suitable root stock clone.

References

- Spurgeon Cox and MA Subair (1999) Performance of the cultivar ATK-1 in Nilgiri-Wynaad. *Newsletter UPASI Tea Research Institute* **9**(2):3-4.
- Mohanam M and VS Sharma (1981) Morphology and Systematics of some tea (*Camellia Spp*) cultivars. *Proceedings of PLACROSYM IV*: 391-400.

Morphological character

Characters	Quantification
Leaf shape	Lanceolate
Total leaf length (cm)	15.2
Lamina length (cm)	14.5
Area of leaf in (mm ²)	4483
Lamina width (cm)	4.7
Ratio between Length and width of Lamina	0.32
Leaf venation	Sunken
Leaf margin	Serrate
Pigmentation in young shoot	Present
Young shoot colour	Mild pink
Individual shoot length (cm)	9.8
Individual shoot weight (g)	1.45
Internodal length (cm)	4.0
Internodal width (cm)	1.4
Stomatal conductance (mmol (H ₂ O) m ⁻² S ⁻¹)	113
Transpiration rate (mmol (H ₂ O) m ⁻² S ⁻¹)	1.95
Net photosynthetic rate ((Co ₂) m ⁻² S ⁻¹)	3.2

*Data presented here is the average value of 4 consecutive years reading.

89. TRI-2025 (IC0636685; INGR21089), a Tea (*Camellia sinensis*) Germplasm Drought Tolerance

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The clone is introduced from Sri Lanka during the 1970's. The clone is popular for its high yield and drought tolerant characters. The genome of these plants has been sequenced under, "Tea genome re-sequencing project".

Morpho-agronomic characteristics: This clone is a diploid with large leaves and grows vigorously throughout the year. Leaves are broadly elliptic or elliptic-oblong and glabrous. The young leaves are light green in colour.

Associated characters and cultivation practices: The clone is preferred for its drought tolerant character as well

as for high yield. It is also a suitable root stock for grafting.

References

- Morphology and Systematics of some tea (*Camellia Spp*) cultivars. *Proceedings of PLACROSYM IV*: 391-400.
- Balasaravanan T, PK Pius and R Rajkumar (2002) Status of south Indian tea germplasm. *Newsletter UPASI Tea Research Foundation*. **12**(2).
- Sasidhar R and R Sanjay (2000) Incidence of collar canker in high range and Anaimallais. *Newsletter UPASI Tea Research Foundation* **10** (1).

Morphological characters

Characters	Quantification
Leaf shape	Elliptic
Total leaf length (cm)	14.0
Lamina length (cm)	13.4
Area of leaf in (mm ²)	5391
Lamina width (cm)	6.0
Ratio between Length and width of Lamina	0.49
Leaf venation	Bullation
Leaf margin	Serrate
Tooth per centimetre	6
Young shoot colour	Green
Individual shoot length (cm)	11.6
Individual shoot weight (g)	1.57
Internodal length (cm)	4.1
Internodal width (cm)	1.5
Stomatal conductance (mmol (H ₂ O) m ⁻² S ⁻¹)	122
Transpiration rate (mmol (H ₂ O) m ⁻² S ⁻¹)	1.86
Net photosynthetic rate ((Co ₂) m ⁻² S ⁻¹)	2.9

*Data presented here is the average value of 4 consecutive years reading.

90. IPC 11A Orange & IPC 11B Orange (IC0635038 & IC0635039; INGR21090), Is The First Orange Colour Main Season Tropical Carrot Carrot (*Daucus carota*) and its CMS Line Developed Indigenously. Roots are of Acceptable Size and Suitable for Main Season Sowing I.E. from Mid-September Onward in North Indian Plains with Petaloid Type Sterility.

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Carrot (*Daucus carota* L.; 2n= 2x=18) is an important vegetable crop being grown worldwide on 1.15 million ha area and production is 42.8 million tonnes (FAOSTAT, 2017). Orange colour carrot is rich in β -carotene, an improvement carotenoid which take care of night blindness. In India, orange carrot is preferred for their high level of uniformity in roots, better storage life, unique taste, and nutritional value. Originally, the orange carrots are temperate (European) type and needs vernalization for flowering and seed production. Hence, most part of the seeds of orange carrot is being imported in country which contributes significantly to foreign seed exchange value. Although, Pusa Nayanjyoti, a cytoplasmic male sterility (CMS) based hybrid was developed by IARI Regional Station, Katrain for main season cultivation but vernalization requirement and limited seed supply limited its spread. Hence, it was essential to develop CMS lines of tropical orange roots (non-vernalization requirement) for establishment of hybrid seed production system in plains. The IPC 11A Orange is an indigenously developed CMS line of tropical carrot which

has orange roots. It is developed by a naturally occurring mutant having 'petaloid' type sterile cytoplasm in Pusa Meghali identified at Division of Vegetable Science, ICAR-IARI, New Delhi. Its maintainer 'IPC 11B Orange' was also searched in the same population having orange self core coloured roots. This CMS line has potential in development of indigenous hybrid seed industry in orange carrot for benefit of local farmers and to improve balance sheet of foreign exchange for vegetable seeds.

Morpho-agronomic characteristics of CMS line IPC 11A Orange

The IPC 11A Orange is the first CMS line of tropical carrot having orange coloured self-core roots developed indigenously. It is suitable for sowing during main crop season (i.e. September to October) and roots are ready to harvest in a period of 90 – 95 days (Table 1). The height of flowering plants was 134.2 cm with profuse flowering and seed setting. The floral traits i.e. petal size, petal colour, petaloid (anther converted petals) colour and size and

nectaries showed normal development (Fig. 1A-B; 2A-B). It produces orange colour, medium long roots with self-core character. Average root length of IPC 11A Orange was observed to be 21.5 cm, root diameter 34.3 mm, core diameter 11.68 mm and root weight (Fig. 3A-B). The marketable root yield was observed to be 27.4 t/ha which was higher than its maintainer line IPC 11A Orange (23.5 t/ha) (Table 1). The bolting (elongation of flower stalk) occurs during February – March months and produce abundant seeds during April month in plains of India. The CMS line showed potential in hybrid combination, hence, has potential in commercial hybrid breeding of indigenous tropical hybrids.

IPC 11B Orange: male fertile maintainer line of CMS line IPC 11A Orange

The 'IPC 11B Orange' is an elite genotype of main season tropical carrot. It is suitable for sowing in September to October in plains of north India. It matures during December to January months after 90-95 days of sowing. Plants are medium vigorous, semi-erect, medium in spread and leaves are green. It produces orange root with self core colour (Table 1).

References

- FAOSTAT (2017) Food and Agriculture Organization, United Nations, Rome accessed on 13 March, 2019.
- NHB Database (2017) Horticultural Statistics at a Glance. National Horticulture Board, Government of India, Gurugaon, India.
- Kalia P, M Mangal, S Singh, C Chugh, M Mishra and S Chaudhary (2019) Morphological and molecular changes on cytoplasmic male sterility (CMS) introgression in Asiatic carrot (*Daucus carota* L.). *Planta* <https://doi.org/10.1007/s00425-019-03185-4>.

Table 1: Important horticultural traits and seed yield of CMS line IPC 11A Orange line as compared to the fertile maintainer line IPC 11B Orange

Traits	IPC 11A Orange	IPC 11B Orange
Maturity traits		
Growing season	Main season	Main season
Sowing period	September – October	September – October
Harvesting	December - January	December - January
Plant and root traits		
Plant height (cm)	85.0	88.0
Gross plant weight (cm)	185	215.0
Root colour	Orange	Orange
Root core colour	Orange	Orange
Root length (cm)	21.5	20.1
Root diameter (mm)	34.3	30.5
Core diameter (mm)	11.68	8.49
Root weight (g)	183.0	156.7
Root yield (t/ha)	27.4	23.5
Floral traits		
Petal colour	White	Green
Petal length (cm)	0.75	0.97
Petaloid colour		-
Light purple		-
Petaloid length (cm)	1.21	-
Petaloid width (cm)	0.77	-
Petaloid shape	Spoon	-
Nectary development	Prominent	Prominent

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