Vol. 36 No. 2 2023

Print ISSN: 0971-8184
Online ISSN: 0976-1926

Indian Journal of Plant Genetic Resources

An International Journal for Conservation and Use of Plant Diversity

Indian Society of Plant Genetic Resources
New Delhi, India

INDIAN SOCIETY OF PLANT GENETIC RESOURCES

(Registration No. S/18336)

The Society was founded in 1987 with the following objectives:

- To serve and promote the scientific cause and to advance academic interest in the field of plant genetic resources.
- To disseminate knowledge relating to various aspects on plant genetic resources.
- To provide a forum for organizing symposia/conferences with a view to develop close relationship among the scientists engaged and interested in plant genetic resources activities.

Indian Journal of Plant Genetic Resources, the official publication of the Society, is published thrice in a year. The contribution to the journal, except for invited papers, is open to the members of the society only.
Membership to the society is open to all the individuals/institutions interested in various aspects of plant genetic resources. The membership fee is as follows:

Membership*	Inland		Foreign	
Life Member	Rs.	5,000	US$	1,500
Annual Member	Rs.	1,000	US$	100
Institutional (Annual)	Rs.	20,000	US$	1,000
Institutional (5 Years)	Rs.	90,000	US$	4,500
Institutional (10 Years)	Rs.	1,75,000	US$	8,500

Issues of the journal published earlier are also available.

Limited space is available for advertisement of interest to botanists/geneticists/plant breeders and all those concerned with plant genetic resources.

CORRESPONDENCE relating to membership, advertisement and other related matters should be addressed to the General Secretary, Indian Society of Plant Genetic Resources, ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110 012, India (E-mail: ispgr2015@gmail.com).

COMMUNICATIONS regarding the publication of research papers should be addressed to
Editor-in-Chief, Indian Journal of Plant Genetic Resources, Indian Society of Plant Genetic Resources, C/o ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110 012, India
(E-mail: ispgr2015@gmail.com).

ISSN: 0971-8184
Online ISSN: 0976-1926

Indian Journal of Plant Genetic Resources

Vol. 36 No. 2 2023

INDIAN SOCIETY OF PLANT GENETIC RESOURCES
NBPGR CAMPUS, NEW DELHI-110012, INDIA
Web: ispgr.nbpgr.ernet.in

INDIAN SOCIETY OF PLANT GENETIC RESOURCES

Patrons	MS Swaminathan, RS Paroda, Mangala Rai
Honorary Fellows	MS Swaminathan, GS Khush, S Rajaram, RS Paroda, Mangala Rai, Emile Frison, RB Singh, SK Datta, HY Mohan Ram, KS Gill, SK Vasal, Kamal Bawa

EXECUTIVE COUNCIL (2022-24)

President	RS Paroda (New Delhi)		
Vice Presidents	RK Tyagi (New Delhi); Jai Chand Rana (New Delhi)		
General Secretary	Anuradha Agrawal (New Delhi)		
Joint Secretary	Manjusha Verma (New Delhi)		
Treasurer	Sanjeev Kumar Singh (New Delhi)		
Ex Officio Member	GP Singh, Director, ICAR-NBPGR		
Councillors	North Zone:	Monika Singh (New Delhi)	Kuldeep Tripathi (New Delhi)
	East Zone:	Mohan Lal (Jorhat)	Veerendra Kumar Verma (Umiam)
	West Zone:	Sandip K. Bera (Junagadh)	Anil Patidar (Jaisalmer)
	South Zone:	N. Sivaraj (Hyderabad)	Rose Mary Francies (Thrissur)
	Central Zone:	Shailesh Tiwari (Varanasi)	Surendra Kumar Barpete (Madhya Pradesh)

CONTENTS

RESEARCH ARTICLE

SHORT COMMUNICATION

BOOK REVIEW

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2022.00036.2.01

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Assessment of Genetic Diversity in Elephant Foot Yam [*Amorphophallus paeoniifolius* (Dennst.) Nicolson var. *campanulatus* (Decne.) Sivad.]

A.K. Choudhary[1*], S. B. Mishra[2] and Shanti Bhushan[3]

Abstract

Wide range of genetic variability was observed in morphological characters like the surface of the pseudo stem, pseudo stem color, leaf spot, stalk of the leaflet, nature of rachis, shape of rachis, color of corm surface top, the color of corm surface bottom and color of flesh while there are no variation were observed for traits *viz*; the number of secondary partition/primary partition, number of primary partition/secondary partition, leaflet spot and stalk of a leaflet. Out of 18 accessions, only two accessions *viz;* Selection -2 and C3 were found to be diverse type in respect to traits viz; surface of pseudo stem and corm shape which having tubercled pseudo stem, respectively. The thickness of the pseudo stem base had also exhibited a highly significant correlation with the fresh weight of corm/plant, height of the corm and diameter of corm. Leptokurtic and positive skewness were exhibited for traits weight of cormel/plant and diameter of corm it indicating that these traits are governed by dominant-based complementary gene action and involving fewer numbers of genes having an increasing effect in the inheritance of these traits. The highest loaded variables in PC1, PC2, PC3 and PC4 were pseudo stem height (cm), weight of cormel /plant (g), length of primary partition (cm) and diameter of corm (cm), respectively. Tuber character i.e., diameter of corm as well as foliage characters, especially pseudo stem height and length of primary partition, is an important trait in distinguishing various accessions of elephant foot yam.

Keywords: Elephant foot yam, Accessions, Principle component analysis, Skewness, Kurtosis and Correlation coefficient.

[1*]Department of Plant Breeding & Genetics, Bhola Paswan Shastri Agricultural College, Purnea, Bihar, India.

[2]Department of Plant Breeding & Genetics, Tirhut College of Agriculture, Dholi, Muzaffarpur, Bihar, India.

[3]Department of Plant Breeding and Genetics, VKSCOA (BAU, Sabour), Dumraon, Bihar, India.

***Author for correspondence:**

anujraubau@gmail.com

Received: 05/07/2021 **Revised:** 06/01/2023

Accepted: 09/01/2023

How to cite this article: Choudhary, A.K., Mishra, S.B., Bhushan, S. (2023). Assessment of Genetic Diversity in Elephant Foot Yam [*Amorphophallus paeoniifolius* (Dennst.) Nicolson var. *campanulatus* (Decne.) Sivad.]. *Indian J. Plant Genetic Resources*. 36(2), 200-207. **DOI:** 10.5958/0976-1926.2022.00036.2.01

Introduction

Elephant foot yam [Amorphophallus *paeoniifolius* (Dennst.) Nicolson var. *Campanulatus* (Decne.) Sivad] was considered for a detailed morphological characterization mainly due to its economic importance. Elephant foot yam is chiefly valued for its edible tuber and profound medicinal properties (Hanelt and IPK 2001); (Ochse and Van der Brink 1980). Elephant foot yam has its distribution in Madagascar eastwards via India to *Malesia*, southern China, Indo-China, Polynesia, northern Australia Hetterscheid and Ittenbach (1996); Lebot (2009). Nicolson (1987) has identified the cultivated form as elephant foot yam. In India, it is cultivated in the state like West Bengal, Andhra Pradesh, Tamil Nadu, Bihar, Chattisgarh, Karnataka, Kerala, Jharkhand, Madhya Pradesh, Jammu & Kashmir and produced about 816.75 m tons (National Horticulture Board, 2021-22).

The corms of elephant foot yam are rich source of carbohydrates and minerals like calcium and phosphorus. The corm is used as a vegetable as well as for the preparation of chips. The corms and cormels of elephant foot yam are usually boiled or baked and eaten as vegetables. The sprouts and petioles, which resemble asparagus sprouts,

are used as vegetables in some parts of Asia (Misra *et al.*, 2007). Genetic variability of breeding materials may lead to the success of the crop improvement program (Prabhu *et al.* 2009). Selection through morphological characters greatly influences on the success of the crop improvement program (Prabakaran 2010). The application of PCA tool provides a useful means for estimating morphological diversity within and between germplasm (Maji and Shaibu 2012). The present study is carried to find out the genetic diversity and selection of elite lines from the existing variability of elephant foot yam.

Materials and Methods

The present investigation was conducted at Tirhut College of Agriculture (Bihar) without replication each plot measuring 14.25 x 1.5 meters in size, row to row as well as plant to plant distance were kept at 0.75 and comprising eighteen elephant foot yam accessions, each of them planted in pit. According to the passport data, these accessions are cultivated in nature and have some ethnobotanical uses (Table 1). Observations were recorded on five randomly selected plants at full growth stage based on elephant foot yam descriptors developed by ICAR-National Bureau of Plant Genetic Resources for elephant foot yam (Abraham *et al.* 2006) for 14 morphological qualitative and 16 quantitative characters (Tables 2 and 3), respectively. The morphological qualitative traits were converted into descriptor scores used for comparing variance analysis with quantitative traits. For the selection of some elite genotypes combining multiple traits with each other on certain limits *viz*;. pseudo stem height (70.66–30.00 cm), length of primary partition (22.67–12.00cm), breath of primary partition (10.00–7.00), no. of leaflets per primary partition (70.00–17.00), length of largest leaflet (14.33–11.20 cm), breath of largest leaflet (6.00–4.00 cm), thickness of pseudo stem base (20.00–12.00 cm), fresh weight of corm/plant (3.50–1.20 kg), height of corm (13.00–9.00 cm) and diameter of corm (40.00–14.00 cm) in excel software. Yield is dependent upon a number of component characters which is quantitatively inherited and considerably affected by the environment. Therefore, direct and indirect selection for yield may not be effective. The selection efficiency under such circumstances can be improved by considering other component characters. Component characters care combined together into a score or index and selection is applied to the index Hazel (1943) and Choudhary *et al.* (2020). All qualitative traits were categorized in different grouped based on the frequency distribution of germplasm in the excel software and principal coordinate analysis (PCoA) for quantitative traits were analysed in SPSS software, whereas correlation

Table 1: Passport data of 18 accessions of elephant foot yam and their accession number

Name of the accessions	*Mission code*	*Accession number*	*Collectors number*	*Particulars*	
C1	O20120003Z08	593138	TCAEFY-1	Species Name (Botanical)	*Amorphophallus paeoniifolius*
C2	O20120003Z08	593139	TCAEFY-2	Common name (English)	Oal, Suran
C3	O20120003Z08	593140	TCAEFY-3	Cultivar/Vernacular name	Elephant foot yam
C4	O20120003Z08	593141	TCAEFY-4	Source	Institution/market/ farmers field
C5	O20120003Z08	593142	TCAEFY-5	Status	Cultivated
C6	O20120003Z08	593143	TCAEFY-6	Frequency	Frequent
C7	O20120003Z08	593144	TCAEFY-7	Material collected	Tuber
C8	O20120003Z08	593145	TCAEFY-8	Sample type	Individual plant
C9	O20120003Z08	593146	TCAEFY-9	Sample method	Random
C10	O20120003Z08	593147	TCAEFY-10	Habitat	Cultivated
C11	O20120003Z08	593148	TCAEFY-11	Disease symptoms	leaf virus
Deshi	O20120003Z08	593149	TCAEFY-12	Insect/Nematode	termite
Trivendrum	-	-	-	Cultural practices	Irrigated
Selection -1	-	-	-	Season	Spring-Summer
Santra Gachhi	-	-	-	Associated crops	Sole
Gajendra	-	-	-	Agronomic scope	Good
BCA -1	-	-	-	Ethnobotanical uses	Yes
Selection -2	-	-	-	Parts	Root
				Kind	Food

Table 2: Frequency (%) distribution of fourteen qualitative characters of elephant foot yam

Sl. No.	*Characters*	*Descriptor score adopted*	*No. of accessions*	*Frequency (%)*
1.	Number of primary partition	3	18	100
2.	Number of secondary partition/primary partition	6	18	100
3.	Number of primary partition/secondary partition	12	18	100
4.	Leaflet nature	1(entire)	18	100
5.	Leaflet margin	2 (undulate)	18	100
6.	Surface of pseudo stem	1 (smooth)	7	38.89
		2 (tubercled)	1(Selection -2)	5.56
		3(spiny)	10	55.56
7.	Pseudo stem color	1(green mottled)	8	44.44
		2(dark greenmottled)	10	55.56
8.	Leaf spot	0 (absent)	0	0
9.	Stalk of leaflet	0 (absent)	0	0
10.	Nature of rachis	1(winged)	11	61.11
		2 (not winged)	9	50.00
11.	Corm shape	1 (depressed)	1(C3)	5.56
		2(flat and globose)	17	94.44
12.	Color of corm surface top	1(black)	4	22.22
		2 (dark brown)	14	77.78
13.	Color of corm surface bottom	1(black)	4	22.22
		2(dark brown)	2(C4 and C10)	11.11
		3 (white)	12	66.67
14.	Color of flesh	1(light yellow)	9	50.00
		2(dark yellow)	7	38.88
		3(reddish yellow)	2 (C11 and Selection -2)	11.11

Table 3: Selection of superior lines by combination of different traits at certain multiple traits range limit

Sl. No.	*Traits*	*Selection Criteria of elite line with multiple traits range limit*	*Selected lines*
1.	Pseudo stem height (cm)	70.66–30.00	C1, C4, C5, C7, C8 and BCA-1
2.	Length of primary partition (cm)	22.67–12.00	
3.	Breath of primary partition (cm)	10.00–7.00	
4.	No. of leaflets per primary partition	70.00–17.00	
5.	Length of largest leaflet (cm)	14.33–11.20	
6.	Breath of largest leaflet (cm)	6.00–4.00	
7.	Thickness of pseudo stem base (cm)	20.00–12.00	
8.	Fresh weight of corm/plant(kg)	3.50–1.20	
9.	Height of corm (cm)	13.00–9.00	
10.	Diameter of corm (cm)	40.00–14.00	

Table 4: Descriptive statistics of different traits of elephant foot yam accessions

Sl. No.	*Traits*	*Mean*	*Range*	*Standard deviation*	*Std. error*	*Skewness*	*Kurtosis*
1.	Pseudo stem height (cm)	49.27	33.00–70.66	11.79	1.68	0.61	-1.00
2.	Length of primary partition (cm)	13.96	8.73–22.66	3.13	0.84	1.25	2.81
3.	Breath of primary partition (cm)	7.59	5.23–9.50	1.12	0.41	-0.33	0.04
4.	No. of leaflets per primary partition	37.15	11.66–79.66	24.42	4.01	0.77	-1.10
5.	Length of largest leaflet (cm)	12.44	11.16–14.33	0.93	0.26	0.40	-0.75
6.	Breath of largest leaflet (cm)	5.09	4.16–6.02	0.55	0.24	0.01	-1.01
7.	Thickness of pseudo stem base (cm)	15.67	11.00–25.00	4.06	1.03	0.89	-0.23
8.	Fresh weight of corm/plant(kg)	1.75	0.83–3.63	0.81	0.62	1.14	0.42
9.	Height of corm (cm)	10.96	8.83–14.33	1.34	0.41	0.97	1.03
10.	Thickness of cormel (cm)	5.52	0.00–10.50	3.45	1.47	-0.54	-0.53
11.	Weight of cormel /plant (g)	47.47	0.00–250.00	68.27	9.91	2.37	5.06
12.	Length of cormel (cm)	2.67	0.00–8.16	2.21	1.35	0.91	0.97
13.	Diameter of corm (cm)	18.75	13.50–80.33	15.42	3.56	4.19	17.71

- coefficient as well as skewness and kurtosis analysis were performed by using online OP stat.

Results and Discussion

Frequency Distribution of Major Qualitative Traits

Vegetative characters

Characterization of 18 elephant foot yam accessions had been done based on its morphological traits such as number of secondary partition/primary partition, number of primary partition/secondary partition, leaflet nature, leaflet margin, the surface of the pseudo stem, pseudostem color, leaf spot, a stalk of a leaflet, nature of rachis, shape of rachis, the color of corm surface top, color of corm surface bottom, corm shape and color of flesh and other, quantitative traits which helps effective utilization of germplasm in crop improvement programmes.

Wide range of genetic variability were observed in morphological characters like the surface of the pseudo stem, pseudo stem color, leaf spot, the stalk of a leaflet, nature of rachis, shape of rachis, color of corm surface top, color of corm surface bottom and color of flesh while there are no variation were observed for traits viz; number of secondary partition/primary partition, number of primary partition/secondary partition, leaflet spot and stalk of a leaflet. Out of 18 accessions, only two accessions *viz;* Selection -2 and C3 were found to have diverse type in respect to traits viz; surface of pseudo stem and corm shape which having tubercled pseudo stem, respectively (Table 2). Thus, the present study suggested that these traits may be useful for the identification of various morphotypes of elephant foot yam Anil *et al.* (2011).

Variation in Quantitative Traits

Out of 18 accessions, only six superior lines *viz,* (C1, C4, C5, C7, C8 and BCA-1) were selected by fixing the certain limits of each quantitative traits *viz;* pseudo stem height (70.66–30.00 cm), length of primary partition (22.67–12.00cm), a breath of primary partition (10.00–7.00), no. of leaflets per primary partition (70.00–17.00), length of a largest leaflet (14.33–11.20 cm), a breath of largest leaflet (6.00–4.00 cm), thickness of pseudo stem base (20.00–12.00 cm), fresh weight of corm/plant(3.50–1.20 kg), height of corm (13.00–9.00 cm) and diameter of corm (40.00-14.00 cm) (Table 3). These traits may be used full for further selection in a breeding programme. (Choudhary *et al.* 2020).The study on the frequency distribution of traits using skewness and kurtosis helps extract the available information on the nature of gene action (Fisher *et al.*, 1932) and the number of genes controlling the traits (Robson, 1956). Leptokurtic and positive skewness were exhibited for traits like weight of cormel/plant and diameter of corm (Table No. 4) it indicated that these traits governed by dominant-based complementary gene action and involving fewer number of genes having an increasing effect in the inheritance of these traits. Maximizing the genetic gain in respect of these traits required intense selection from the existing variability. The correlation coefficient is a statistical measure to determine the extent of association, whether positive or negative, between various plant characters, which help to identify the character on which selection can be imposed for improvement in associated characters (Table 5). Pseudo stem height had exhibited highly significant correlation with breath of primary partition, no. of leaflets per primary partition, thickness of pseudostem, height of corm and diameter of corm. Breath of primary partition was also found to have a highly significant correlation with no. of leaflets per primary partition, pseudostem base thickness and corm height. The thickness of the pseudostem base had also exhibited highly significant correlation with the fresh

Table 5: Correlation coefficient among qualitative traits of the elephant foot yam accessions

Sl. No.	*Traits*	*Pseudo stem height (cm) Recorded at full foliage*	*Length of primary partition (cm)*	*Breath of primary partition (cm)*	*No. of leaflets per primary partition*	*Length of largest leaflet (cm)*	*Breath of largest leaflet (cm)*	*Thickness of pseudo stem base (cm)*	*Fresh weight of corm/ plant(kg)*	*Height of corm (cm)*	*Thickness of cormel (cm)*	*Weight of cormel/ plant (g)*	*Length of cormel (cm)*	*Diameter of corm (cm)*
1.	Pseudo stem height (cm) recorded	1.000	0.282	0.833**	0.808**	-0.113	0.088	0.903**	0.474*	0.742**	-0.559*	-0.458	-0.412	0.503*
2.	Length of primary partition (cm)		1.000	0.352	-0.153	0.217	0.412	0.098	-0.072	0.163	0.202	-0.114	0.122	0.006
3.	Breath of primary partition (cm)			1.000	0.742**	0.053	0.056	0.768**	0.273	0.698**	-0.403	-0.385	-0.296	0.152
4.	No. of leaflets per primary partition				1.000	-0.251	-0.359	0.900**	0.337	0.593**	-0.577*	-0.195	-0.300	0.333
5.	Length of largest leaflet (cm)					1.000	0.540*	-0.247	-0.167	-0.246	-0.126	-0.350	-0.230	-0.211
6.	Breath of largest leaflet (cm)						1.000	-0.117	0.075	0.042	-0.125	-0.469*	-0.342	0.251
7.	Thickness of Pseudo stem base (cm)							1.000	0.513*	0.696**	-0.517*	-0.211	-0.280	0.621**
8.	Fresh weight of corm/plant (kg)								1.000	0.535*	-0.276	-0.236	-0.226	0.605**
9.	Height of corm (cm)									1.000	-0.229	-0.365	-0.179	0.443
10.	Thickness of cormel (cm)										1.000	0.688**	0.880**	-0.403
11.	Weight of cormel/ plant (g)											1.000	0.883**	-0.176
12.	Length of cormel (cm)												1.000	-0.293
13.	Diameter of corm (cm)													1.000

Table 6: Mean values of 13 characters of the five clusters for the 18 elephant foot yam germplasm.

Cluster	Pseudo stem height (cm) Recorded at full foliage	Length of primary partition (cm)	Breath of primary partition (cm)	No. of leaflets per primary partition	Length of largest leaflet (cm)	Breath of largest leaflet (cm)	Thickness of pseudo stem base (cm)	Fresh weight of corm/plant (kg)	Height of corm (cm)	Thickness of cormel (cm)	Weight of cormel/ plant (g)	Length of cormel (cm)	Diameter of corm (cm)
I	33.00	8.73	5.23	26.00	11.23	4.16	11.83	.83	8.83	9.83	200.00	6.00	13.83
II	64.11	13.00	9.13	74.33	12.33	4.62	20.38	2.03	12.44	2.28	7.22	1.39	16.66
III	60.66	22.66	8.66	48.00	12.00	4.90	19.16	1.50	11.66	10.06	86.66	5.50	15.50
IV	38.66	13.33	7.50	38.00	11.83	4.43	16.33	1.70	10.16	10.50	250.00	8.16	16.00
V	43.36	14.33	7.16	19.40	12.80	5.36	12.94	1.61	10.60	6.22	29.61	2.43	14.61

Table 7: Distribution of elephant foot yam germplasm in different cluster groups

Cluster	*Number of germplasm*	*Name of the germplasm*
I	10	C9, Santra Gachhi (14), C8, C5, C11, C1, C4, C10, C6 and Selection-2(17)
II	01	C2
III	04	Selection -1(13), Gajendra (13), BCA -1(16), Ttrivendrum (collected from Trivendrum 12)
IV	01	C3
V	02	C7 and Deshi (18)

weight of corm/plant, height of corm and diameter of corm. Indicating that these traits may be given more importance for further improvement in breeding programmes. Similar findings were corroborated by Abraham *et al.* (2008) and Anil *et al.* (2011).

Cluster analysis of variance due to accessions of all the traits were found to be highly significant except for the trait i.e. weight of corm/plant, which indicated a sufficient amount of variability present in the population. Mean values of 13 characters of the five clusters for the 18 elephant foot yam germplasm (Table 6).

The maximum number of accessions falls under cluster I (10) followed by cluster III (4) and the minimum number in cluster II and IV (1) (Table 7). The maximum cluster mean value was recorded for traits i.e. weight of cormel/plant (g) in cluster I, III and IV whereas in cluster II and V for traits number of leaflets per primary partition and pseudo stem height (cm), respectively (Table 6).

PCA is a well-known method of dimension reduction that can be used to reduce a large set of variables to a small set that still contains most of the information in the large set Massay (1965) and Jolliffie (1986). The result of the PCA explained the genetic diversity of the elephant foot yam genotypes (Table 8). The PC4 accounted maximum cumulative variability (85.37%) among all the components which had been contributed by trait like diameter of corm (cm) followed by fresh weight of corm/plant. Similarly PC3 component had shared (75.35%) of the variability with dominance foliage characters such as length of primary partition (cm) and breath of largest leaflet (cm) as well as tuber traits viz; height of corm (cm), thickness of cormel (cm) and length of cormel (cm). The PC2 contributed for (62.31%) of the variability by the characters such as weight of cormel/plant (g), length of cormel (cm), number of leaflets per primary partition and thickness of cormel (cm). PC1 had contributed minimum variability (42.56%) for foliage traits *viz*; pseudo stem height (cm) breath of primary partition, no. of leaflets per primary partition, thickness of pseudo stem base (cm) and height of corm (cm). The highest loaded

Table 8: Principal components for 13 yield contributing traits of elephant foot yam

Traits	*Principal components*			
	PC1	*PC2*	*PC3*	*PC4*
Pseudo stem height (cm)	0.937	0.095	0.211	-0.099
Length of primary partition (cm)	0.104	-0.234	0.867	-0.053
Breath of primary partition (cm)	0.793	0.062	0.365	-0.408
No. of leaflets per primary partition	0.813	0.399	-0.154	-0.333
Length of largest leaflet (cm)	-0.084	-0.755	0.183	-0.198
Breath of largest leaflet (cm)	0.116	-0.773	0.363	0.361
Thickness of pseudo stem base (cm)	0.900	0.34	0.086	-0.025
Fresh weight of corm/plant (kg)	0.587	0.145	-0.039	0.586
Height of corm (cm)	0.765	0.237	0.287	0.097
Thickness of cormel (cm)	-0.722	0.351	0.505	0.129
Weight of cormel/plant (g)	-0.589	0.691	0.145	0.079
Length of cormel (cm)	-0.602	0.612	0.451	0.057
Diameter of corm (cm)	0.603	0.074	-0.052	0.684
Eigen value	5.533	2.568	1.691	1.306
Percentage of variation	42.565	19.753	13.009	10.043
Cumulative (%)	42.565	62.318	75.327	85.370

Table 9: Proximity dissimilarity matrix (euclidean distance) between elephant foot yam accessions

Cluster	*I*	*II*	*III*	*IV*	*V*
I	00.00	201.684	119.864	52.301	171.035
II		00.00	84.799	247.080	63.528
III			00.00	165.440	67.149
IV				00.00	221.374
V					00.00

variables in PC1, PC2, PC3 and PC4 were pseudo stem height (cm), weight of cormel/plant (g), length of primary partition (cm) and diameter of corm (cm), respectively. Hence tuber character i.e. diameter of corm as well as foliage characters, especially pseudo stem height and length of primary partition, is an important trait in distinguishing various accessions of elephant foot yam. Similar findings corroborated with Anil *et al.* (2011) and Singh *et al.* (2020).

Cluster Analysis and Dissimilarity Matrix of Elephant foot Yam Accessions

Dendrogram (Ward's method) for 18 accessions of elephant foot yam using 13 morphological traits which were represented in Figure 1. Dendrogram represented the fairly and simply interpreted relationship among the accessions. The horizontal axis of the dendrogram represented the dissimilarity. The vertical axis represents the objects and clusters. Each joining (fusion) of two clusters on the graph is represented by splitting a horizontal line into two. The

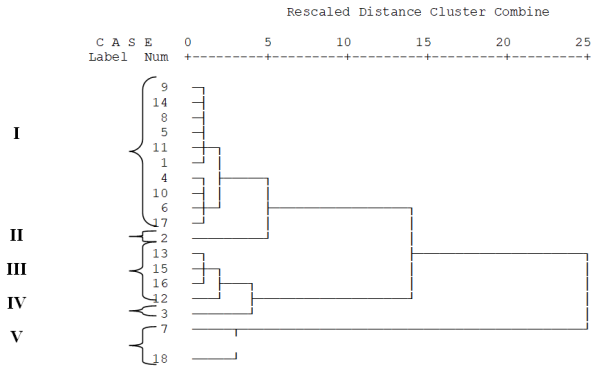

Figure 1: Dendrogram showing hierarchical clusters of 18 elephant foot yam accessions (UPGMA) based on quantitative characters

horizontal position of the split, shown by the short vertical bar, gives the distance (dissimilarity) between the two clusters. C7 and Deshi were in cluster-V while C3 was found in cluster-IV. The dissimilarity analysis recorded highest inter-cluster distance between cluster II and IV (247.08) followed by cluster IV and V (221.37). The lowest cluster distance was exhibited between cluster I and IV (52.30) (Table 9). Genetic diversity evaluation can be used full for selecting promising genotypes. Similar finding reported by Shekar *et al.* (2012); Kumar *et al.* (2013); Arivalagan *et al.* (2013). Touhiduzzaman *et al.* (2016), Sikder *et al.* (2015), Mehraj *et al.* (2017), Rahim *et al.* (2010), and Hoque and Rahman (2007) showed that hybrid genotypes with maximum inter-cluster distance resulted in high yielding, the crossing between these genotypes can be used in breeding programmes to

achieve maximum heterosis. Hence, it may be concluded that a wide range of genetic variability were observed for qualitative and quantitative traits which might be useful for further breeding program me or it may be directly used in cultivation. Tuber character i.e. diameter of corm as well as foliage characters, especially pseudo stem height and length of primary partition, is an important trait in distinguishing various accessions of elephant foot yam.

Acknowledgment

Highly thankful AICRP on tuber crops (other than potato) who have given financial support for conducting the trial.

References

Abraham Z, Latha M, Asha KI, Varghese C., Lakhminarayan S, Pareek SK (2006) Minimal descriptors of agri-horticultural crops Part V: spices, tubers and plantation crops, NBPGR, Regional station, Thrissur, pp 102.

Abraham Z, Latha M, Brinda R (2008) Character association studies in Elephant foot yam *J. root. crops.* **34:** 70–72.

Anil Shirly Raicha, Siril EA, Beevy S Suhara (2011) Morphological variability in 17 wild Elephant foot yam (Elephant foot yam paeoniifolius) collections from southwest India. *Genet. Resour. Crop Evol.* **58:** 1263–1274.

Arivalagan M, Bhardwaj R, Gangopadhyay KK, Prasad TV and Sarkar SK (2013) Mineral composition and their genetic variability analysis in eggplant (*Solanum melongena*L.) germplasm. *J. Appl. Bot. Food.Qual.* **86:** 99-103

Choudhary AK, Mishra SB, Choudhary VK, Bhushan Shanti and Singh AK (2020) Morpho-Physiological Diversity in Arvi (*Colocasia esculenta* (L.) Schott.Var. *Antiquorum*). *Int. J. Curr. Microbiol. App. Sci,* **9(6):** 3551-3560.

Fisher RA, Immer FR, Tedin (1932) The genetical interpretation of statistics of the third degree in the study of quantitative inheritance. *Genetics.* **17:** 107 –124.

Hanelt P (2001) Institute of Plant Genetics and Crop Plant Research Mansfeld's encyclopedia of agricultural and horticultural crops, vol 6. *Springer-Verlag*, 2317–2340.

Hazel, LM (1943) The genetic basis for constructing selection indices. *Genetics*. **28:** 476-490.

Hetterscheid WLA, Ittenbach S (1996) Everything you always wanted to know about Elephant foot yam but were afraid to stick your nose into. *Aroideana.* **19:** 7–151.

Hoque MN and Rahman L (2007) Estimation of euclidean distance for different morpho-physiological characters in some wild and cultivated rice genotypes (*Oryza sativa* L.). *J. Biol. Sci.*, **7(1):** 86-88.

Jolliffie IT (1986) Principal Component Analysis. *Springer,* New York.

Kumar, SR (2013) Arumugam T. and Anandakumar C. R.: Genetic diversity in egg plant (*Solanum melongena*L.). *Plant Gene Trait*, **4(2):** 4-8.

Lebot V (2009) Tropical root and tuber crops: cassava, sweet potato, yams and aroids. *Crop production science in horticulture* 117.

Maji AT and Shaibu AA (2012) Application of principal component analysis for rice germplasm characterization and evaluation. *J. Plant Breed. Crop Sci.* **4(6):** 87-93.

Massay WF (1965) Principal components regression in exploratory statistical research. *J. Am. Stat. Assoc.* **60:** 234-246.

Mehraj Hasan and Shimasaki Kazuhiko (2017) Hierarchical cluster, euclidean distance and principal component analysis based on phenotypic characters of hosta. *J. Biosci. Agric. Res.* **12(02):** 1029-1035.

Misra RS, Nedunchezhiyan M, Acharya M and Rana singh N (2007) Post-harvest management of Elephant foot yam tubers, in: *Root and Tuber Crops: Post-harvest Management and Value Addition* (eds G. Padmaja, T. Premkumar, S. Edison and B. Nambisan), *Proceedings of the National Seminar on Achievements and Opportunities in Post-harvest Management and Value Addition in Root and Tuber Crops (NSRTC2)*, Central Tuber Crops Research Institutem, Thiruvananthapuram, Kerala, India: pp, 150–154.

Nicolson DH Araceaeln (1987) Dassanayake MD, Fosberg FR (eds) A *revised Handbook to the flora of Ceylon*, **VI**, 17–101.

Ochse JJ,Van der Brink RCB (1980) Vegetables of the Dutch East Indies. Amsterdam.

Prabakaran S (2010) (Evaluation of local types of brinjal (*Solanum melongena* L.) M. Sc., (Hort.) Thesis: Agricultural College and Research Institute, TNAU, Madurai.

Prabhu M, Natarajan S and Pugalendhi L (2009) Variability and heritability studies in F5 and F6 progenies of brinjal. *Sustain. Agric. AEJSA*. **3(3):** 306-309.

Rahim MA, Mia AA, Mahmud F, Zeba N, Afrin K (2010) Genetic variability, character association and genetic divergence in mungbean. *Plant Omics* **3(1):** 1-6.

Robson DS (1956) Application of K4 statistics to genetic variance component analysis. *Biometrics.* **12:** 433–444.

Shekar CK, Ashok P and Sasikala K (2012) Studies on heritability and multivariate analyses in brinjal (*Solanum melongena* L.). *Veg. Crop. Res. Bull.*, **76:** 79-88.

Sikder RK, Rahman MA, Asif MI, Jamal Uddin AFM and Mehraj H (2015) Genetic variability, distance and traits interrelationship analysis of Nerica and Inpari rice varieties. *Sci. Agric.* **10(1):** 44-48.

Singh Pragya, Jain PK and Tiwari Akanksha (2020) Principal Component Analysis Approach for Yield Attributing Traits in Chilli (*Capsicum annum* L.) Genotypes. *Chem. Sci. Rev. Lett*, **9(33):** 87-91.

Touhiduzzaman Sikder, RK Asif MI, Mehraj H and Jamal Uddin AFM (2016) Correlation and genetic distance on sixteen rice varieties grown under SRI. *Adv. Plants Agric. Res.*, **3(3):** 100.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2022.00036.2.02

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Genetic Diversity Studies for Yield and Yield attributing Characters in Colored Sorghum Genotypes

Kiran[1], Suvarna[1], B. V. Tembhurne[1], G. Girish[2], Shivaleela[1] and M. Lakshmikanth[1]

Abstract

The experiment was carried out at the College of Agriculture, Raichur, during *rabi* 2020. It was undertaken to assess the nature of genetic variability and diversity among 120 colored sorghum genotypes. The study revealed wide variation for yield and yield attributing traits, high GCV and PCV coupled with high heritability and genetic advance was observed for neck of panicle, peduncle length, 100-grain weight. The traits *viz.,* plant height, neck of panicle, peduncle length, panicle length, 100-grain weight, panicle weight and grain yield per plant showed high heritability and genetic advance as percent of mean. Wide genetic diversity was observed among the genotypes as evidenced by the formation of seven clusters for the 120 colored genotypes based on different traits studied. Out of ten characters studied, contribution of plant height towards genetic divergence was the highest followed by panicle weight.

Keywords: Colored sorghum, Diversity, Genotypic coefficient of variation, Phenotypic coefficient of variation, Yield.

[1]College of Agriculture, University of Agricultural Sciences, Raichur, Karnataka, India.

[2]Agriculture Research Station, Raichur, Karnataka, India.

***Author for correspondence:**

suvarnagpb@uasraichur.edu.in

Received: 17/03/2022 **Revised:** 04/07/2023

Accepted: 05/07/2023

How to cite this article: Kiran, Suvarna, Tembhurne, B.V., Girish, G., Shivaleela, Lakshmikanth, M. (2023). Genetic Diversity Studies for Yield and Yield attributing Characters in Colored Sorghum Genotypes. *Indian J. Plant Genetic Resources*. 36(2), 208-215. **DOI:** 10.5958/0976-1926.2022.00036.2.02

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench], popularly called as jowar, is the "king of millets" or "Great Millet" and is the fifth most important cereal crop in the world after rice, wheat, maize and barely, in terms of production and utilization. The word sorghum is derived from the latin word "Sorgo" which means "raising above". It is known as a failsafe crop and camel of crops because of its drought tolerance, heat tolerance, and high photosynthetic efficiency. So, it is considered as an important staple food crop in arid and semi-arid regions of the world (Anagholi *et al*., 2000). Sorghum is originated in Africa. It is an often cross-pollinated, diploid (2n=20) and C_4 grass plant species, which belongs to the family "Graminae" and tribe "Andropogeneae". Cultivated sorghum has five basic races, *viz*., bicolor, durra, guinea, caudatum and kafir and ten intermediate races.

In India, during the period of 2017 to 2021, sorghum had an average area of 2.93 m ha with an average production of 2.55 million tones and the average productivity was 872 kg/ha. Correspondingly in Karnataka during the same period, the average area of sorghum was 0.80 m ha with an average production of 0.79 million tonnes and the productivity was 996 kg/ha (Anon., 2022).

Sorghum grain contains different pericarp colors *viz*., white, yellow, brown, purple, red, black, etc. These colored sorghum grains vary with respect to nutritional contents. White sorghum has low levels of total phenolic contents and has very low levels or zero levels of tannin

and 3-deoxyanthocyanidin (Awika and Rooney, 2004). Yellow sorghum is rich in flavanones and has slightly higher total phenolic contents than white sorghum (Dykes *et al*., 2011). Red sorghum has moderately high levels of phenolic compounds but lacks tannins. Black sorghum is genetically red and is a special type of red sorghum because the red pericarp changes into black under sunlight radiation during maturation. Black sorghum has high levels of phenolic contents that are concentrated in the pericarp, particularly the content of 3-deoxyanthocyanidins (Dykes *et al*., 2009 and 2013). Brown sorghum, also known as tannin sorghum, has pigmented testa and high levels of condensed tannins (Awika and Rooney, 2004).

So, developing genotypes with high yield potential coupled with nutritionally superior quality grains is the prime objective of the breeding programme. Improvement in grain yield and quality depends on the nature and extent of genetic variability, heritability and genetic advance in the base population along with information on the nature of association between yield and its components helps in simultaneous selection for many characters.

Genetic diversity in the crop species is one of the precious gifts of nature. Phenotypic traits are conventional tools to analyse genetic diversity. The genetic variability of cultivated crops and their wild relatives together form a potential and continued source for breeding new and better crop varieties, a better understanding of the genetic diversity in colored sorghum would greatly contribute to crop improvement with a view of grain nutritional quality and other important agronomic traits. Therefore, there is a need to evaluate the available accessions for genetic diversity in the colored sorghum genotypes and identify the best accessions according to their performance.

Understanding the wealth of genetic diversity in sorghum will facilitate further improvement of this crop for its genetic architecture. This study aims to determine the genetic variation and diversity present in the colored sorghum genotypes.

Material and Methods

The experimental material comprised of 120 colored sorghum genotypes. The indigenous and exotic collections were obtained from R.S. Paroda Gene-bank, ICRISAT, Patancheru. The five checks used in the study were M 35-1, Paiyur 2, AKJ 1, IS 2312 and DJ 6514.

M 35-1, IS 2312 and DJ 6514 were obtained from ARS, Hagari, UAS, Raichur, AKJ 1 was obtained from RARS, Vijayapur, UAS, Dharwad and Paiyur 2 was obtained from TNAU, Coimbatore. The list of genotypes used for the study is presented in Table 1.

The genotypes along with five checks were sown on 30-10-2020 (*rabi,* 2020) in an augmented design. Each entry was sown in a single line. The checks were replicated in 3 blocks. Each block was of 4 m length with uniform spacing

Table 1A: List of colored sorghum genotypes with country origin and checks used in the present study

Sl. No.	*Genotype*	*Country*
1	IS 2502	United states of America
2	IS 2582	United states of America
3	IS 2618	United states of America
4	IS 3817	Mali
5	IS 3579	Sudan
6	IS 522	Mexico
7	IS 6508	India
8	IS 7013	Sudan
9	IS 7527	Nigeria
10	IS 8222	Uganda
11	IS 8792	Zimbabwe
12	IS 9664	Sudan
13	IS 9667	Sudan
14	IS 11180	Ethiopia
15	IS 12643	Ethiopia
16	IS 14094	South Africa
17	IS 14897	Cameroon
18	IS 14904	Cameroon
19	IS 14905	Cameroon
20	IS 15098	Cameroon
21	IS 16006	Cameroon
22	IS 16169	Cameroon
23	IS 16202	Cameroon
24	IS 16310	Cameroon
25	IS 16316	Cameroon
26	IS 16398	Cameroon
27	IS 17591	Yemen
28	IS 18301	Niger
29	IS 18639	Nigeria
30	IS 18679	United States of America
31	IS 19298	Sudan
32	IS 19299	Sudan
33	IS 19498	Sudan
34	IS 20301	Niger
35	IS 20842	United states of America
36	IS 21835	Sudan
37	IS 21868	Yemen
38	IS 22436	Sudan
39	IS 22897	Sudan

40	IS 22942	Sudan
41	IS 22949	Sudan
42	IS 22970	Sudan
43	IS 23864	Yemen
44	IS 23865	Yemen
45	IS 23890	Yemen
46	IS 23916	Yemen
47	IS 23950	Yemen
48	IS 23953	Yemen
49	IS 23954	Yemen
50	IS 23955	Yemen
51	IS 24001	Yemen
52	IS 25040	Sudan
53	IS 28000	Yemen
54	IS 28001	Yemen
55	IS 28009	Yemen
56	IS 28014	Yemen
57	IS 28015	Yemen
58	IS 28017	Yemen
59	IS 28049	Yemen
60	IS 28050	Yemen
61	IS 28056	Yemen
62	IS 28065	Yemen
63	IS 28074	Yemen
64	IS 28172	Yemen
65	IS 28176	Yemen
66	IS 28198	Yemen
67	IS 28200	Yemen
68	IS 28202	Yemen
69	IS 28210	Yemen
70	IS 28217	Yemen
71	IS 28224	Yemen
72	IS 28230	Yemen
73	IS 28237	Yemen
74	IS 28243	Yemen
75	IS 28244	Yemen
76	IS 28250	Yemen
77	IS 28265	Yemen
78	IS 28791	Yemen
79	IS 28792	Yemen
80	IS 28966	Yemen
81	IS 28982	Yemen
82	IS 29012	Yemen
83	IS 29013	Yemen
84	IS 29014	Yemen
85	IS 29031	Yemen
86	IS 29032	Yemen
87	IS 29033	Yemen
88	IS 29052	Yemen
89	IS 29055	Yemen
90	IS 30722	Cameroon
91	IS 30736	Cameroon
92	IS 30754	Cameroon
93	IS 30781	Cameroon
94	IS 30800	Cameroon
95	IS 30802	Cameroon
96	IS 31706	Yemen
97	IS 31718	Yemen
98	IS 31731	Yemen
99	IS 31732	Yemen
100	IS 31906	Yemen
101	IS 32072	Yemen
102	IS 32079	Yemen
103	IS 32121	Yemen
104	IS 32163	Yemen
105	IS 32165	Yemen
106	IS 32185	Yemen
107	IS 33158	Cameroon
108	IS 33159	Cameroon
109	IS 33310	Cameroon
110	IS 33317	Cameroon
111	IS 33323	Cameroon
112	IS 33336	Cameroon
113	IS 33343	Cameroon
114	IS 34723	Cameroon
115	IS 35642	Chad
116	IS 35823	Russian Federation
117	IS 35838	Russian Federation
118	IS 38527	Ethiopia
119	IS 39564	Ethiopia
120	IS 40175	Mauritania

Figure 1B: Checks used in the present

Sl. No.	*Check*
1	M 35-1
2	AKJ 1
3	Paiyur 2
4	IS 2312
5	DJ 6514

of 45 cm between rows and 15 cm between plants. All the necessary package of practices and need-based plant protection measures were followed to raise healthy crop. Observations were recorded for the characters *viz*., days to 50% flowering, days to maturity, plant height (cm), neck of panicle (cm), peduncle length (cm), panicle length (cm), panicle width (cm), 100-grain weight (g), panicle weight (g) and grain yield per plant (g). Observations were taken on randomly selected five plants in each genotype and average of this was recorded as mean data for each character in each genotype (Plate 1 and 2).

Results and Discussion

The genetic variability parameters *viz*., genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad sense heritability (h^2) and genetic advance as percent of mean (GAM) were computed to know the extent of genetic variability for ten characters and presented in Table 2.

Phenotypic coefficient variation (PCV) and genotypic coefficient variation (GCV) values were high for grain yield per plant (53.37 and 45.02%) followed by neck of panicle (50.53 and 49.92%), panicle weight (45.24 and 43.45%), peduncle length (35.05 and 31.39%), panicle width (32.93 and 24.73%) and 100-grain weight (27.06 and 26.49%), respectively. This indicates that the material showed more

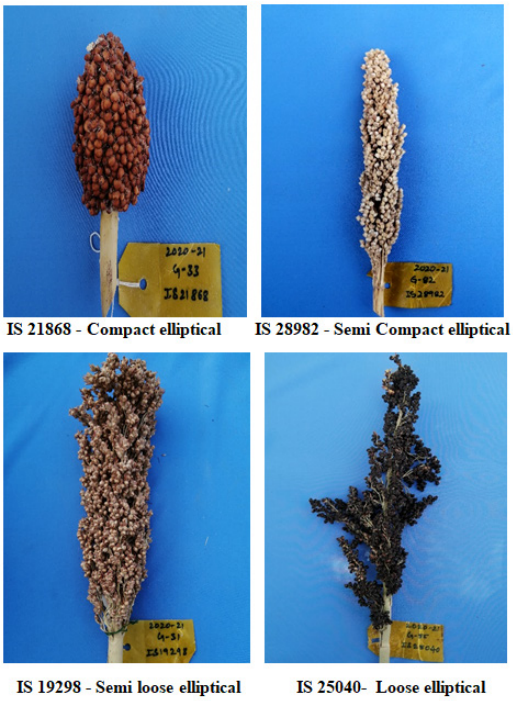

Plate 1: Variation for inflorescence compactness and shape in colored sorghum genotypes.

Plate 2: Variation for grain color in sorghum genotypes

Table 2: Genetic variability parameters for yield and yield attributing characters

Sl. No.	*Character*	*Co-efficient of variation*		*Heritability (%) broad sense*	*Genetic advance as per cent over mean (GAM)*
		Genotypic Coefficient of Variation GCV (%)	*Phenotypic Coefficient of Variation PCV (%)*		
1	Plant height (cm)	14.58	16.27	80.31	26.91
2	Days to 50 % flowering	04.42	04.72	87.96	08.54
3	Days to maturity	03.20	03.66	76.60	05.76
4	Neck of panicle (cm)	49.92	50.53	97.61	101.60
5	Peduncle length (cm)	31.39	35.05	80.18	57.89
6	Panicle width (cm)	24.73	32.93	56.38	38.24
7	Panicle length (cm)	15.56	19.13	66.17	26.07
8	100-Grain weight (g)	26.49	27.06	95.81	53.41
9	Panicle weight(g)	43.45	45.24	92.25	85.96
10	Grain yield per plant (g)	45.02	53.37	71.14	78.21

variation for these characters. The PCV and GCV values were moderate for plant height (16.27 and 14.58%) and panicle length (19.13 and 15.56%).

Similar results of high PCV and GCV for grain yield per plant, panicle length and panicle weight were reported by Khandelwal *et al.* (2015) and Swamy *et al.* (2018), for plant height and panicle length by Sushil (2014), for plant height and grain yield per plant by Arunkumar (2013) and moderate PCV and GCV for 100-grain weight, days to maturity and days to 50% flowering by Yaqoob *et al.* (2015), moderate to high PCV and GCV for days to 50% flowering, grain yield, 100-grain weight and plant height by Yohannes *et al.* (2015).

The lowest PCV and GCV values were exhibited for days to maturity (3.66 and 3.20%) and for days to 50% flowering (4.72 and 4.42%). This indicates that the studied genotypes showed less variation for these two characters. The genotypes were selected for the study based on the different seed colors and exotic. Majority of the accessions were from Cameroon and Yemen. This may be the reason for less variation in these two characters studied.

The PCV values were higher than their respective GCV values for the characters like peduncle length, panicle width, panicle weight, panicle length, plant height and grain yield per plant. It means that the apparent variation is not only due to genotypes but also due to the influence of the environment. The characters neck of panicle, 100-grain weight, days to 50% flowering and days to maturity had little difference between PCV and GCV, indicating that their variation has a genetic origin that could be exploited for further breeding programmes.

Heritability value in broad sense is presented in Table 2. The highest heritability was recorded for the characters *viz.*, neck of panicle (97.61%) followed by 100-grain weight (95.81%), panicle weight (92.25%), grain yield per row (91.01%), days to 50% flowering (87.96%), plant height (80.31%), peduncle length (80.18%), days to maturity (76.60%), grain yield per plant (71.14%) and panicle length (66.17%). This indicates that these characters showed high heritability and selection for these characters could be effective. Moderate heritability was exhibited for the character panicle width (56.38%). High heritability for the characters like grain yield per row, days to 50% flowering and plant height was reported by Khandelwal *et al.* (2015), Yaqoob *et al.* (2015) and Yohannes *et al.* (2015), for characters days to maturity, panicle length and panicle weight was reported by Bello *et al.* (2007) and Khandelwal *et al.* (2015).

The maximum genetic gain percent over a mean (GAM) was recorded for neck of the panicle (101.60%) followed by grain yield per row (98.31%), panicle weight (85.96%), grain yield per plant (78.21%), peduncle length (57.89%), 100 grain weight (53.41%), panicle width (38.24%), plant height (26.91%) and panicle length (26.07%). It indicates that additive genes govern the character and selection will be rewarding for the improvement of such trait. Whereas minimum GAM was recorded for days to 50% flowering (8.54%) and days to maturity (5.76%), which indicates that the character is governed by non-additive genes and heterosis breeding may be useful. Similar results of high GAM for the character panicle length and grain yield per plant were reported by Santosh *et al*. (2013), for grain yield per plant and plant height by Yaqoob *et al.* (2015) and for days to 50% flowering and plant height by Yohannes *et al.* (2015).

High heritability along with genetic advance as percent of mean was recorded for traits *viz.*, neck of panicle (97.61 and 101.60%) followed by 100-grain weight (95.81 and 53.41%), panicle weight (92.25 and 85.96%), grain yield per plot (91.01

Table 3: Grouping of colored sorghum genotypes based on D2 analysis

Cluster	*No. of Entries*	*Genotype*
I	113	IS 16398, IS 28000, IS 23865, IS 19498 IS 3310, IS 28243, IS 22970, IS 28074, IS 28210, IS 33343, IS 23950, IS 28014, IS 28049, IS 28001, IS 28009, IS 29014, IS 32072, IS 32121, IS 28200, IS 40175, IS 28050, IS 2312, Paiyur-2, IS 23916, IS 7527, IS 33159, IS 28202, IS 30781, IS 14904, IS 28237, IS 28230, IS 28172, IS 38527, IS 20301, IS 28791, IS 6508, IS 32163, IS 22949, IS 39564, IS 18679, IS 30736, IS 16202, IS 28966, IS 32079, M-35-1, IS 28056, IS 29055, IS 3579, IS 19298, IS 28792, IS 9667, IS 20842, IS 30800, IS 31731, IS 35823, IS 28224, IS 23953, IS 2582, IS 14897, IS 16316, IS 32165, IS 29031, DJ 6514, IS 522, IS 30754, IS 29012, IS 28017, IS 29052, IS14905, IS 7013, IS 16169, IS 28015, IS 2502, IS 32185, IS 30722, IS 31906, IS 25040, IS 2618, IS 14094, IS 31706, IS 35838, IS 29033, IS 23864, IS 8792, IS 18301, IS 3817, IS 28176, AKJ-1, IS 9664, IS 35642, IS 8222, IS 28244, IS 28198, IS 29013, IS 34723, IS 33336, IS 21835, IS 22897, IS 19299, IS 28065, IS 15098, IS 28982, IS 28265, IS 30802, IS 33317, IS 23955, IS 23890, IS 22436, IS 22942, IS 28250, IS 33323, IS 28217, IS 21868
II	3	IS 23954, IS 33158, IS 29032
III	4	IS 31718, IS 31732, IS 18639, IS 17591
IV	1	IS 16310
V	1	IS 12643
VI	1	IS 11180
VII	2	IS 16006, IS 24001

Table 4: Average Intra and inter cluster distances for eleven characters in colored sorghum genotypes

Cluster	*Cluster I*	*Cluster II*	*Cluster III*	*Cluster IV*	*Cluster V*	*Cluster VI*	*Cluster VII*
Cluster I	3889.87	20816.49	12964.75	12631.64	13079.64	10429.78	17525.44
Cluster II		2806.34	31705.34	18894.47	18046.66	37026.45	13424.02
Cluster III			3963.56	40117.58	40795.39	32700.21	22103.53
Cluster IV				0.00	1609.40	6784.53	26521.46
Cluster V					0.00	10037.09	22865.98
Cluster VI						0.00	35024.05
Cluster VII							3865.86

Diagonal values indicate intra cluster distances
Above diagonal values indicate inter cluster distances

Table 5: Cluster means for eleven characters in colored sorghum genotypes

Cluster	*PH*	*DFF*	*DM*	*NP*	*PEDL*	*PWD*	*PL*	*100GW*	*PW*	*GYPP*	*Overall score*	*Rank*
Cluster I	207.58 (5)	76.32 (4)	110.76 (3)	23.41 (3)	49.60 (5)	5.12 (4)	22.69 (4)	4.26 (3)	55.40 (5)	37.85 (4)	40	3
Cluster II	225.27 (4)	76.67 (3)	109.33 (4)	21.40 (5)	47.20 (6)	5.74 (2)	20.87 (6)	5.60 (1)	157.67 (2)	122.50 (1)	34	5
Cluster III	110.60 (7)	75.50 (5)	109.25 (5)	21.60 (4)	50.45 (4)	3.23 (7)	16.50 (7)	3.00 (6)	65.00 (6)	35.88 (5)	56	1
Cluster IV	303.20 (2)	70.00 **(7)**	108.00 **(7)**	38.20 (2)	70.04 (2)	5.50 (3)	26.80 (3)	4.60 (2)	72.00 (4)	62.00 (2)	34	6
Cluster V	306.00 (1)	78.00 (2)	111.00 (2)	5.10 **(7)**	55.20 (3)	4.10 (6)	28.80 (2)	3.80 (5)	83.75 (3)	53.75 (3)	34	7
Cluster VI	272.00 (3)	74.00 (6)	109.00 (6)	46.50 (1)	92.20 (1)	5.00 (5)	32.00 (1)	2.40 (7)	21.00 (7)	11.00 (7)	44	2
Cluster VII	196.00 (6)	78.50 (1)	116.50 (1)	16.35 (6)	28.20 (7)	6.75 (1)	21.50 (5)	3.90 (4)	172.50 (1)	22.50 (6)	38	4

PH= Plant height (cm) DFF = Days to 50 % flowering DM = Days to maturity NP= Neck of panicle (cm)
PEDL= Peduncle length (cm) PWD=Panicle width (cm) PL= Panicle length (cm) 100GW=100 Grain weight (g) PW=Panicle weight (g) GYPP= Grain yield per plant (g)
Value in parenthesis indicates the ranking of genotypes for each character separately

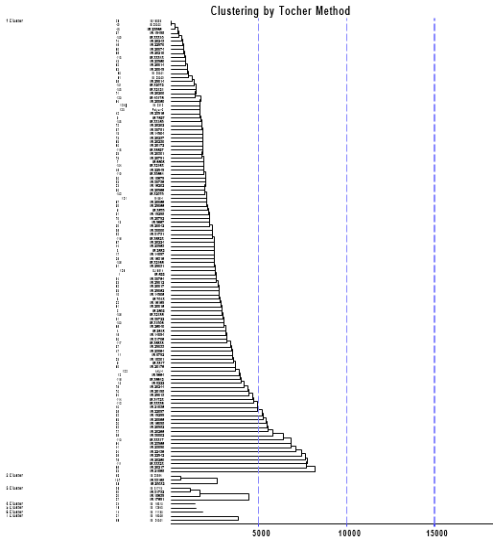

Fig. 1: Cluster diagram of colored sorghum genotypes

and 98.31%), plant height (80.31 and 26.91%), peduncle length (80.18 and 57.89%), grain yield per plant (71.14 and 78.21%) and panicle length (66.17 and 26.07%). This indicates that there was low environmental influence and greater role of genetic component of variation, which shows that additive gene action present in these traits. The high value of additive gene effects indicates higher breeding value, so the selection of these characters is effective for desired genetic improvement. Similar findings were also observed by Deepalakshmi and Ganesamurthy (2007), Nyadanu and Dikera (2014), Santosh *et al*. (2013) and Sushil (2014).

Genetic Diversity

The present investigation was undertaken to study the genetic divergence in the color sorghum genotypes using the D^2 analysis given by Mahalanobis (1936). D^2 is one of the reliable methods to understand the genetic diversity present

Table 6: Percent contribution of each character towards divergence in colored sorghum genotypes

Sl. No.	*Character*	*Per cent contribution*	*Times ranked 1st*	*Cumulative contribution*
1	Plant height	43.81	3395	43.81
2	Panicle weight	25.47	1974	69.28
3	Peduncle length	15.63	1211	84.91
4	Grain yield per plant	10.31	799	95.22
5	Neck of panicle	4.41	342	99.63
6	Panicle length	0.14	11	99.77
7	Days to 50% flowering	0.13	10	99.90
8	Days to maturity	0.10	8	100.00
9	Panicle width	0.00	000	000
10	100 grain weight	0.00	000	000

in the genotypes using Tocher's method. Estimating genetic diversity within and between germplasm groups is vital and useful for properly selecting parents to perceive higher heterosis and get potential segregants. Genetic diversity analysis has been used to quantify (a) the genetic distance between the genotypes (b) to identify divergent genotype to initiate the crossing programme (c) to know clustering pattern of genotypes.

Group Constellation

In the present investigation, D^2 was applied to all the genotypes studied, which classified the total genotypes into seven clusters (Table 3 and Figure 1). The analysis of cluster pattern revealed that, the highest number of genotypes are present in cluster I (113), followed by cluster III (4), cluster II (3) and cluster VII (2). The cluster IV, V, and VI had a solitary one. The distribution pattern of genotypes into various clusters was at random, suggesting that genetic diversity was unrelated to geographic diversity. The results are in accordance with Rohman *et al*. (2004), Sameer *et al.* (2010), Shinde *et al*. (2013) and Kavya *et al*. (2019).

Intra and Interrelation of Clusters

The average intra and inter-cluster distances for the ten characters in colored sorghum genotypes are presented in Table 4. Inter-cluster distances were higher than the intra-cluster distances. Suggesting that, wide genetic diversity existed among the genotypes of different groups. The intra-cluster average D^2 values were ranged from 0 (cluster IV, V and VI) to 3963.56 (Cluster III). The highest intra-cluster distance (3963.56) was observed in cluster III, followed by cluster I (3889.87) and cluster VII (3865.86), indicating that wide genetic variation was present among the genotypes within these clusters. So, more emphasis will be given for the genotypes belonging to these clusters during selection of parents for hybridization programme.

Cluster II (2806.34) had moderate intra-cluster distance. The lowest intra-cluster distance (0) was observed in clusters IV, V, VI which are monogenotypic (IS 16310), (IS 12343), (IS11180), respectively and were divergent from genotypes belonging to other clusters.

The inter-cluster D^2 values was ranged from 1609.40 (between cluster IV and cluster V) to 40795 (between cluster III and cluster V). The maximum inter-cluster distance was observed between cluster IV and cluster V (40795), followed by between cluster III and cluster IV (40117.58), between cluster II and cluster VI (37026.45) and between cluster VI and cluster VII (35024.05), revealing that genotypes included in these clusters are genetically diverge and may give rise to desirable genotypes in the segregating generation. The lowest inter-cluster distance was observed between cluster IV and cluster V (1609.40) followed by cluster IV and cluster VI (6784.53), indicating that these clusters' genotypes were genetically least diverse and almost of the same genetic architecture. The results are in accordance with Prasad and Biradar (2017), Ahalawat *et al*. (2018) and Kavya *et al*. (2019).

Cluster means

The cluster means and overall score value for ten characters in colored sorghum genotypes was presented in Table 5. The character days to 50 % flowering showed cluster mean value ranged from 70 (cluster IV) to 78.50 days (cluster VII). Cluster IV genotypes showed characteristic early flowering habit with mean number of days to flowering was 74 days. These clusters comprised of early flowering genotypes. While genotypes of cluster VII showed late flowering habit with mean number of days to flowering was 78.50 days. The genotypes with early flowering (Cluster IV) are preferred over late flowering because they mature early and the crop duration will be less.

Cluster VI had highest mean value for panicle length (32.00 cm), cluster VII for panicle width (6.75 cm) and panicle weight (172.50 g), cluster II for 100-grain weight (5.20 g) and grain yield per plant (122.50 g). The promising genotypes from these clusters with high mean values for different characters may be used directly for adaption or as parents in hybridization programme to achieve high yield levels.

In cluster IV, the lowest cluster mean values were observed for the characters days to 50% flowering and days to maturity. So, selecting genotypes belonging to these clusters helps develop short-duration varieties. The lowest cluster mean values for plant height (110.60 cm), neck

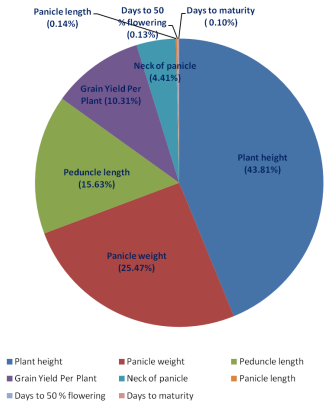

Figure 2: Contribution of major characters towards genetic diversity in colored sorghum genotypes

of panicle (5.10 cm) and peduncle length (28.20 cm) was exhibited by cluster III, V, and cluster VII, respectively, so the selection of genotypes from this cluster, helps in developing varieties with short plant height.

Contribution of Different Characters towards Divergence

Percent contribution of characters towards genetic divergence was analyzed and presented in Table 6 and Figure 2. The analysis revealed that, plant height alone contributed 43.81% to the divergence. Among yield attributing characters, panicle weight contributed 25.47% to total divergence, followed by peduncle length (15.63%), grain yield per plant (10.31%), neck of panicle (4.41%). Panicle length, days to 50% flowering, and days to maturity showed less percent of contribution towards total divergence. Whereas the characters, panicle width and 100-grain weight were not contributed to the total genetic divergence. Similar results were also reported by Rani and Rao (2012), Rekha *et al*. (2013) and Prasad and Biradar (2017).

Out of ten characters studied, four characters *viz*., plant height, panicle weight, peduncle length and grain yield per plant contributed 95.22% to the total genetic divergence. These characters should be given more importance in further breeding programmes.

References

Ahalawat NK, Arya VK, Kumar P, & Singh SK. (2018). Genetic divergence in forage sorghum [*Sorghum bicolor* (L.) Moench]. *J. Nat. Appl. Sci.,* **10(1):** 439-444.

Anagholi A, Kashiri A, & Mokhtarpoor H. (2000). The study of comparison between forage sorghum cultivars and speed feed hybrids. *J. Agric. Nat. Resour. Sci.,* **7(4):** 73-83.

Anonymous, (2022). India sorghum production and utilization. New Delhi. *http://www.indiastat.com/agriculture/2/foodgrains/17180/jowargreatmillet.*

Awika, J. M., & Rooney, L.W. (2004). Sorghum phytochemicals and their potential impact on human health. *Phytochemistry,* **65(9):** 1199–1221.

Dykes, L., Peterson, G. C., Rooney, W. L., & Rooney, L. W. (2011). Flavonoid composition of lemon-yellow sorghum genotypes. *Food Chem.,* **128(1):** 173–179.

Dykes, L., Rooney, W. L., & Rooney, L. W. (2013). Evaluation of phenolics and antioxidant activity of black sorghum hybrids. *J. Cereal Sci.,* **58(2):** 278–283.

Dykes, L., Seitz, L. M., Rooney, W. L., & Rooney, L. W. (2009). Flavonoid composition of red sorghum genotypes. *Food Chem.* **116(1):** 313–317.

Deepalakshmi, A. J., & Ganesamurthy, K. (2007). Studies on genetic variability and character association in *kharif* sorghum [*Sorghum bicolor* (L.) Moench]. *Indian J. Agric. Res.,* **41(3):** 177-182.

Kavya, P., Rao, V. S., Vijayalakshmi, B., Sreekanth, B., Radhakrishna, Y., & Umar, S. N. (2019). Genetic divergence study in sorghum (*Sorghum bicolor* L.) using D^2 analysis. *Intl. J. Curr. Microbiol. App. Sci.,* **8(6):** 24-31.

Nyadanu, D., & Dikera, E. (2014). Exploring variation, relationships and heritability of traits among selected accessions of sorghum [*Sorghum bicolor* (L.) Moench] in the upper east region of Ghana. *J. Pl. Breed. Genet.,* **2(3):** 101-107.

Prasad, B., & Biradar, B. D. (2017). Genetic diversity studies in minicore collection of *rabi* sorghum using D^2 statistics. *Intl. J. Curr. Microbiol. App. Sci.* **6(7):** 850-856.

Rani, J. K., & Rao, S. S. (2012). Genetic diversity in sorghum (*Sorghum bicolor*) for panicle components and morpho-physiological traits important for *rabi* adaptation. *Crop Res., **44*(1 & 2):** 156-160.

Rekha, C., Biradar, B.D., Salimath, P. M., & Sajjanar, G. M. (2013). Genetic divergence for grain quality and productivity traits in *rabi* sorghum. *Intl. J. Agric. Innov. Res.,* **1(5):** 132-135.

Rohman, M.M., Hakim, M.A., Sultana, N. A., Kabir, M. E., Hasanuzzan, M., & Ali, M. (2004). Genetic divergence analysis in sorghum. *Asian J. Pl. Sci.* **3(2):** 211-214.

Sameer, K. C. V., Shreelakshmi, & Shivani, D. (2010). Genetic diversity analysis in *rabi* sorghum [*Sorghum bicolor* (L.) Moench] local genotypes. *Electron. J. Pl.,* **1(4):** 527-529.

Santosh, K., Girish, G., Dharmaraj, P.S., Lokesha, R., & Jayalakshmi, S. K. (2013). Evaluation of sorghum landraces for charcoal rot resistance and yield traits in *rabi* sorghum. *M. Sc. (Agri) Thesis submitted to the Electron. J. Pl., Raichur,* Karnataka, India.

Shinde, D., Smita, C., & Jadhav, B. D. (2013). Study of genetic divergence in sweet sorghum [*Sorghum bicolor* (L.) Moench]. *BioScan.* **8(1):** 135-138.

Sushil, K. (2014). Genetic variability in land races of forage sorghum [*Sorghum bicolor* (L.) Moench] collected from different geographical origin of India. *Prime Res. on Med.,* **3(2):** 146-153.

Swami, S.S., Chaudhary, S. B., & Kute, N. S. (2015). Genetic diversity studies in *rabi* sorghum [*Sorghum bicolor* (L.) Moench]. *J. Agric. Res. Technol.* **40(2):** 203-207.

Yohannes, T., M., Weldetsion, N., Abraha, Manyasa, E., & Abraha, T. (2015). Combine selection for earliness and yield in pedigree developed sorghum [*Sorghum bicolor* (L.) Moench] progenies in Eritrea. *J. Pl. Breed. Genet.,* **3(1):** 01-08.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2022.00036.2.03

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Genetic Relationships and Population Structure among Maize (*Zea mays* L.) Landraces as Revealed by Simple Sequence Repeat Markers

S.K. Singh, Shipra Deo, S.B. Chaudhary, Kuldeep Singh and Mukesh K. Rana*

Abstract

A set of 48 maize (*Zea mays* L.) genotypes, including landraces, some hybrids and inbreds, was characterized using 34 simple sequence repeat markers distributed throughout the genome. The 34 SSR primers produced bands in the range of 2 to 6 with an average of 3.2 bands per primer. The size range of these bands varied from 90 to 310 bp. Genetic similarity calculated using Jaccard's coefficient varied from 0.122 to 0.750 with a mean of 0.475. Gene diversity or expected heterozygosity varied from 0.208 to 0.805, averaging 0.502. The average PIC was 0.433, with a range of 0.078 to 0.776. The range of resolving power was from 0.17 to 2.15 and marker index ranged from 0.16 to 4.83. Cluster analysis using the unweighted pair group method with arithmetic mean showed two major clusters with minor sub-clusters. The first three principal coordinates accounted for 26.7% of the total variation. Principal coordinates analysis and population structure aided in further elucidation of the genetic relationships as well as differentiation of genotypes. Analysis of variance revealed 81.7% within population variation and 18.3% between population variation. The analysis also led to the identification of specific and highly informative SSR markers, namely BNLG 1182, BNLG 1175, UMC 1353, BNLG 1159, BNLG 1272 and BNLG 1045, which significantly contributed to the differentiation of the material.

Keywords: Genetic diversity, Landraces, Population structure, SSR markers, *Zea mays* L.

ICAR-National Bureau of Plant Genetic Resources, New Delhi, Delhi, India.

***Author for correspondence:**

Mukesh.Rana@icar.gov.in

Received: 16/04/2022 **Revised:** 06/02/2023

Accepted: 16/02/2023

How to cite this article: Singh, S.K., Deo, S., Chaudhary, S.B., Singh, K., Rana, M.K. (2023). Genetic Relationships and Population Structure among Maize (*Zea mays* L.) Landraces as Revealed by Simple Sequence Repeat Markers. *Indian J. Plant Genetic Resources*. 36(2), 216-226.

DOI: 10.5958/0976-1926.2022.00036.2.03

Introduction

Maize (*Zea mays* L.) is one of India's most dynamic crops with wider adaptability under varied agro-climatic conditions with an area of 9.86 mha and productivity of 3195 kgha^{-1} during 2020-21 (GOI, 2021). In addition to staple food for human being and quality feed for animals, it serves as a basic raw material as an ingredient to thousands of industrial products that includes starch, oil, protein, alcoholic beverages, food sweeteners, pharmaceutical, cosmetic, textile, gum, package and paper industries *etc*. Maize landraces represent a rich source of variation and must be properly characterized for efficient management, particularly for efficient nutrient uptake and utilization, as well as for useful genes for adaptation to stressful environments. The morphological and physiological variation of maize is evidenced by its large number of landraces being grown in India. Landraces are dynamic populations with a historical origin, and distinct identity, often genetically diverse and locally adapted, and associated with a set of farmers' practices of seed selection and field management as well as with traditional knowledge.

Various kinds of marker techniques, such as morphological, biochemical and DNA-based, have been used to study genetic diversity and population structure

analysis in maize. Morphological assessment is usually the first step in this direction; however, DNA-based markers are nowadays preferred because these are not influenced by environmental factors or by the plant growth stage. DNA-based markers such as restriction fragment length polymorphisms (RFLPs) (Zehr *et al.*, 1992), random amplified polymorphic DNAs (RAPDs) (Lahane *et al.*, 2016; Berhitu *et al.*, 2019), amplified fragment length polymorphisms (AFLPs) (Roy and Kim, 2016), inter- simple sequence repeats (ISSRs) (Dar *et al.*, 2018) and single nucleotide polymorphism (SNPs) (Boakyewaa Adu *et al.*, 2019; Tomkowiak *et al.*, 2019) markers have been used extensively in various genetic analyses in maize.

SSR markers (Tautz, 1989) are the markers of choice due to their many advantages over the others because they are PCR based, abundant and dispersed throughout a genome; highly mutagenic, polymorphic, and informative; co-dominant, suitable for detecting heterozygotes, and multi-allelic; experimentally reproducible; transferable among related taxa; cost-effective and easy to detect; amplified from low quality and low quantity of DNAs; and presumably neutral (Abdurakhmonov, 2016). SSRs have been used in maize for various applications, including population structure and diversity analysis (Mahar *et al.*, 2009; Bracco *et al.*, 2016; Saiyad and Kumar, 2018; Malik *et al.*, 2020), DNA fingerprinting, discrimination, identification and DUS analysis of varieties (Wang *et al.*, 2002; Gunjaca *et al.*, 2008; Nguyen *et al.*, 2012) heterotic group construction and heterosis performance (Barbosa *et al.*, 2003; Hu *et al.*, 2017; Punya *et al.*, 2019) and core collection construction (Yao *et al.*, 2008). The use of microsatellite markers in maize landrace characterization has also been demonstrated (Qi-Lun *et al.*, 2008; Singode and Prasanna, 2010; Herrera-Saucedo *et al.*, 2019; Rathod *et al.*, 2020; Goyanka *et al.* 2021). In the present study, the SSR markers has been used to reveal the diversity and genetic structure in landraces of maize from Jharkhand.

Materials and Methods

Plant Material and DNA Extraction

In 37 landraces of maize were included in the present study (Table 1). In addition, four hybrids (DHM117, HQPM1, VL Baby Corn 1 and VL Amber Popcorn) and seven inbred lines (BML 7, CM 128, CM 141, CML 32, CM 144, LM 15 and PMC 6) were also studied. The 37 landraces represented four Jharkhand state districts: Jaspur, Korba, Koriya and Kunkuri, with 18, 7, 9 and 1 landraces, respectively. The 40 to 50 seeds of each genotype (sufficient to represent the variability) were raised in paper towels in the laboratory in replicates and 2 to 3 weeks old seedlings were used for DNA extraction. Equal amount of fresh leaf tissue from each seedling was pooled to make a representative sample for each genotype. Total genomic DNA was extracted by following CTAB method (Saghai-Maroof *et al.*, 1984) with minor modifications. The

Table 1: List of maize (*Zea mays* L.) genotypes (landraces, hybrids and inbreds) used for SSR analysis

S. No.	*National Identity/ Name*	*Collector No.*	*Village/Block/District*	*Biological Status*
1	IC624140	SKB/PM-5	Dipatoli/Jaspur/ Jaspur	Landrace
2	IC624141	SKB/PM-6	Dipatoli/Jaspur/ Jaspur	Landrace
3	IC624142	SKB/PM-7	Dipatoli/Jaspur/ Jaspur	Landrace
4	IC624143	SKB/PM-8	Dipatoli/Jaspur/ Jaspur	Landrace
5	IC624144	SKB/PM-9A	Khatanga/ Mahakultoli/Jaspur	Landrace
6	IC624145	SKB/PM-10	Khatanga/ Mahakultoli/Jaspur	Landrace
7	IC624146	SKB/PM-11	Khatanga/ Mahakultoli/Jaspur	Landrace
8	IC624147	SKB/PM-12	Khatanga/ Mahakultoli/Jaspur	Landrace
9	IC624148	SKB/PM-14	Bilaspur/Kunkuri/ Jaspur	Landrace
10	IC624149	SKB/PM-15	Matashi/Kunkuri/ Jaspur	Landrace
11	IC624150	SKB/PM-16	Matashi/Kunkuri/ Jaspur	Landrace
12	IC624151	SKB/PM-17	Deoratoli/Jaspur/ Kunkuri	Landrace
13	IC624153	SKB/PM-19	Rauni/Bagicha/ Jaspur	Landrace
14	IC624154	SKB/PM-21	Budhadand/ Gajupura/Jaspur	Landrace
15	IC624155	Not known	Not known	Landrace
16	IC624157	SKB/PM-28	Chikani pani/ Pathalgaon/Jaspur	Landrace
17	SKB/PM-30	Not known	Not known	Landrace
18	IC624158	SKB/PM-31	Sakdukala/Korba/ Korba	Landrace
19	IC624159	SKB/PM-35	Madanpur/Pali/ Korba	Landrace
20	IC624160	SKB/PM-36	Madanpur/Pali/ Korba	Landrace
21	IC624164	SKB/PM-46	Dubar muddha/Pali/ Korba	Landrace
22	IC624165	SKB/PM-47	Ranwa/Pondi/Korba	Landrace
23	IC624166	SKB/PM-48	Beran/Pondi/Korba	Landrace
24	IC624168	SKB/PM-54	Amjhar/ Pondipurpara/Korba	Landrace
25	IC624169	SKB/PM-56	Devadant/ Khadgaon/Koriya	Landrace

26	IC624170	SKB/PM-58	Pahadpara/Sonhat/Koriya	Landrace
27	IC624171	SKB/PM-61	Turi Pauni/Sonhat/Koriya	Landrace
28	IC624172	SKB/PM-63	Turi Pauni/Sonhat/Koriya	Landrace
29	IC624173	SKB/PM-66	Senghor/Sonhat/Koriya	Landrace
30	IC624174	SKB/PM-71	Chainpur/Manendergarh/Koriya	Landrace
31	IC624175	SKB/PM-73	Mauari/Manendergarh/Koriya	Landrace
32	IC624176	SKB/PM-75	Gutra/Manendergarh/Koriya	Landrace
33	IC624177	SKB/PM-76	Siroli/Manendergarh/Koriya	Landrace
34	IC624178	SKB/PM-77	Siroli/Manendergarh/Jaspur	Landrace
35	IC624179	SKB/PM-78	Rauni/Bagicha/Jaspur	Landrace
36	IC624180	SKB/PM-79	Rauni/Bagicha/Jaspur	Landrace
37	IC624181	SKB/PM-83	Songada/Manora/Jaspur	Landrace
38	DHM117	_	_	Hybrid
39	BML-7	_	_	Inbred line
40	CM128	_	_	Inbred line
41	CM141	_	_	Inbred line
42	CML32	_	_	Inbred line
43	CM144	_	_	Inbred line
44	LM15	_	_	Inbred line
45	PMC6	_	_	Inbred line
46	HQPM1	_	_	Hybrid
47	VL Baby Corn-1	_	_	Hybrid
48	VL Amber Popcorn	_	_	Hybrid

concentration and quality of the isolated genomic DNA was estimated using the Nano-drop spectrophotometer (ND-1000, USA) and a final working concentration of 10 ng μL^{-1} was made and stored at -4°C for further analysis.

PCR Amplification of SSR Markers

Initially, PCR conditions were optimized using different template DNA and $MgCl_2$ concentrations. Each optimized PCR reaction mixture consisted of 10.5 ng template DNA, 1X PCR buffer [10 mM Tris-HCl (pH.8.3), 50 mM KCl] 1.5 mM $MgCl_2$, 0.5 U of *Taq* polymerase, 200 µM of each dNTP (all chemicals from Sigma-Aldrich, USA) and 0.4 µM forward and reverse primers in a total volume of 15 µL. Amplifications were performed in a thermocycler (MJ Research, Model PTC-200) using the following cycling conditions: a denaturation step of 5 minutes at 95°C followed by 40 cycles each composed of 1-minute at 94°C, 1-minute at 55°C and 1-minute at 72°C. A final extension step of 8 minutes at 72°C was run at the end of the last PCR cycle.

Based on their high PIC value and representing the whole genome of maize, 34 SSR primers were used for diversity assessment (Table 2). At the time of electrophoresis, PCR products were mixed with 1-µL of gel loading dye (6x buffer: bromophenol blue, 0.25; xylene cyanol FF, 0.25; and glycerol in water 30%) and spun briefly in a microfuge before loading on to the gel. The amplification products were electrophoresed and visualized on a 3% metaphor-agarose (3:1) gel stained with ethidium bromide. The size of the amplified SSR fragments was estimated by running 100 bp DNA ladder (M/S BR Biochem Life Sciences) in the gel as a standard size marker. After electrophoresis, the gel was photographed in a gel documentation system under UV light.

SSR Data Scoring and Marker Statistics

Amplified fragments of different sizes were considered as different alleles. Only distinct and reproducible bands were scored as present (1) or absent (0) for each SSR primer pair. The scored data thus was recorded in an MS excel spreadsheet and the resultant data matrix was subjected to further analysis. Various marker statistics were calculated which consisted of total number of bands, total number of polymorphic bands, percent polymorphism, number of bands per marker, allelic frequency, gene diversity, heterozygosity, polymorphic information content (PIC) (Botstein *et al.*, 1980), effective multiplex ratio (EMR), resolving power (RP) (Prevost and Wilkinson, 1999), marker index (MI) (Powell *et al.*, 1996) and probability of identity (PI) (Paetkau *et al.*, 1995). Microsoft Excel spreadsheet was used to calculate all these indices.

Genetic Diversity and Genotype Clustering

Computer software NTSYS-pc version 2.2 (Rohlf, 2004) was used to calculate Jaccard's similarity coefficient values and generate a dendrogram to decipher the genetic relationship among the genotypes. For comparing any two genotypes, i_1 and i_2, Jaccard's coefficient was calculated as: a/ (a + b + c), where *a* denotes the number of positions with shared bands (1s) for both individuals; *b* the number of positions where

Table 2: SSR primers used for genetic diversity analysis in maize (*Zea mays* L.) landraces

S. no.	*Primer name*	*Size range (bp)*	*Forward sequence*	*Reverse sequence*
1	BNLG 128	170-200	CACCTGGAGGGACCCATTCC	AGGACCACAGGATCCATCATCCT
2	BNLG 238	160-200	CTTATTGCTTTCGTCATACACACACATTCAT	GAGCATGAGCTTGCATATTTCTTGTGG
3	BNGL 240	130-140	AAGAACAGAAGGCATTGATACATAA	TGCAGGTGTATGGGCAGCTA
4	BNLG 1346	160-210	CATCATGAAGCAATGAAGCC	CCGCGCCATTATCTAGTTGT
5	BNLG 105	120-150	GACCGCCCGGGACTGTAAGT	AGGAAAGAAGGTGACGCGCTTTTC
6	BNLG 615	190-220	CTTCCCTCTCCCCATCTCCTTTCCAA	GCAACCTGTCCATTCTCACCAGAGGATT
7	BNLG 1045	120-160	TCCCCGATAGCATATCGATC	GTGACTTTGGGGAGTTTGGA
8	BNLG 1159	90-190	GTGTGCCTATCCTTCCGAGA	AAGGACGTCAACAACGAACC
9	BNLG 490	100-190	GCCCTAGCTTGCTAATTAACTAACA	ACTGTAAGGGCAGTGGACCTATA
10	BNLG 589	150-160	GGGTCGTTTAGGGAGGCACCTTTGGT	GCGACAGACAGACAGACAAGCGCATTGT
11	BNLG 1154	170-190	GGGTGATCACATGGGTTAGG	AAATCAATGCTCCAAATCGC
12	BNLG 1178	250-310	GCTCATGTGCAAATGCAAGT	ACTACAGTTGAACGCCCCTG
13	BNLG 1182	110-180	AGCCGAGTCAGTTCGAGGTA	CAGGGGCTTGAGGTGAGTTA
14	BNLG 1272	180-250	ACCGAAGATGAGGTGTGACA	TCAGTGCAAGGGCAATTTAG
15	BNLG 1175	120-220	ACTTGCACGGTCTCGCTTAT	GCACTCCATCGCTATCTTCC
16	BNLG 1520	170-200	TCCTCTTGCTCTCCATGTCC	ACAGCTGCGTAGCTTCTTCC
17	BNLG 1190	110-180	ACCTTAGTTACACAGGCACACGGT	GGTGATGGGATTTTCGCATTATTA
18	BNLG 1325	160-180	CTAAATGCGCAGCAGTAGCA	TGCTCTGCAACAACTTGAGG
19	PHI 38920	100-110	ACCTTAGTTACACAGGCACACGGT	GGTGATGGGATTTTCGCATTATTA
20	PHI 116	170-180	GCATACGGCCATGGATGGGA	TCCCTGCCGGGACTCCTG
21	PHI 119	90-180	GGGCTCCAGTTTTCAGTCATTGG	ATCTTTCGTGCGGAGGAATGGTCA
22	UMC 2336	130	ATCTCACCGCACGTAACTGAGACT	CCTATGCTCTTGCTCTTCCTGGTA
23	UMC 2134	150-160	CAGGCGACGAAGATGAATTGAA	TAGTCTAGCGTCGACGAAAAATGC
24	UMC 2165	150-170	AGAACACCAAATGGTGACGTTATGT	CTAGCTCGTCTTCCCTGTGGTCT
25	UMC 1068	120-150	AGTCGTTTTCAAAGGCTGCTGATA	TGAGTCACCTCATTTCTTCTGGTTC
26	UMC 1353	180-230	AGACAGGATCATCGAAAACACACA	ACCTCAGCCTCCTCGTCAACTACT
27	UMC 2017	170-180	AGAGGTTACTACGGAGTGTGGCAG	GTCAGGGTACTGCTTCTCGAACTC
28	UMC 1690	110-120	ACCTTAGTTACACAGGCACACGGT	GGTGATGGGATTTTCGCATTATTA
29	UMC 2043	100-170	AGAGGTTACTACGGAGTGTGGCAG	GTCAGGGTACTGCTTCTCGAACTC
30	UMC 2258	100-110	AAGATTGTATAAATGGCAGCCACG	GAATAAGACCAGACAGCACCGAAC
31	UMC 2325	150-160	CTACGATATCCACCTCTACCACCG	CCTATGCTCTTGCTCTTCCTGGTA
32	UMC 2036	110	TCAATCAAGCCTCTCGTAAGGAAC	CTCTTGATCTCAACCGAAATCCTG
33	UMC 2190	190	GATCCGTTGAGGTCGATCCTTT	GAGGAGTTCCTGCAGTTTCTTGAC
34	UMC 2229	300	CGAAGAGCACGATGTTGACG	GAGAAGGGCGGGAGGAATAAC

individual i_1 has a band, but i_2 does not, and *c* the number of positions where individual i_2 has a band, but i_1 does not. Principal coordinate analysis (PCoA) was also computed using the same NTSYS-pc software.

Calculation of Discrimination Indices

The discrimination power (D_j) of a primer, which describes the probability that two randomly chosen individuals from a set of N individuals have different banding pattern or are distinguishable from one another, was calculated as described by Tessier *et al.* (1999). D_j was calculated from unity by subtracting Cj, i.e., the probability that two randomly chosen individuals have identical banding patterns. The confusion probability for i^{th} pattern with frequency of pi for the given j^{th} primer was calculated as: c_j = , and summing of all such c_js for *l* different patterns generated by a primer gives the confusion probability C_j of that primer. Hence, $D_j = 1 - C_j$ = 1 - . Estimated discrimination power (D_L) for each of the j primers was calculated as an extension of the polymorphism information content (PIC) described by Anderson *et al.* (1993) and is given as: D_L = 1 -Theoretically, the total number of non-differentiated pairs of individuals (x_j) for the j^{th} primer were calculated as: $x_j = (N(N-1)/2)C_j$. For a set of k primers, the total number of non-differentiating pairs of individuals (x_k),

under the hypothesis of independence of the considered primer patterns, were calculated as: $_j$.

AMOVA and Population Structure Analysis

AMOVA (Analysis of MOlecular VAriance) (Excoffier *et al*., 1992) was used to partition the total genetic variation to among- and within population variance components and to calculate genetic variation in each group, using the ARLEQUIN software ver. 3 (Excoffier & Lischer, 2010). Population structure was estimated using a Bayesian Markov Chain Monte Carlo model (MCMC) implemented in STRUCTURE v2.3.4 (Pritchard *et al*., 2000). Five runs were performed for each number of populations (k) set from 1 to 7. Burn-in time and MCMC replication numbers were set to 10,000 and 10,000, respectively for each run, respectively. The most probable K-value was determined by Structure Harvester (Earl and Vonholdt, 2012), using the log probability of the data [LnP(D)] and delta K (ΔK) based on the rate of change in [LnP(D)] between successive K-values. To create a graphical plot of structure, DISTRUCT software was used (Rosenberg, 2004).

Results and Discussion

The domestication of crop plants and modern plant breeding practices have resulted in the loss of crop diversity. In order to retain the allelic richness found in the wild, the resort to landraces is inevitable to sustain genetic diversity. Landraces are not only a valuable source of genetic diversity but are also useful to specific adaptations under local environmental situations, and these can serve as sources of various improved agronomical attributes (Lopes *et al*., 2015). The present study assessed the genetic diversity based on SSR markers in the maize landraces from mainly three districts: Jaspur, Korba and Koriya from the Jharkhand state of India. These landraces were obtained from the Regional Research Station of ICAR-National Bureau of Plant Genetic Resources, Ranchi. In addition, four hybrids and seven inbred lines were also included in the study to compare the genetic variability.

The Usefulness of SSR Markers for Polymorphism Detection

The 34 SSR primers, representing the whole genome of maize used to assess the genetic diversity in the maize landraces and to decipher their genetic relationships, produced very sharp and scorable bands in size range as low as 90 bp pairs (primers BNLG 1159 and PHI 119) to as high as 310 bp (primer BNLG 1178). The size range and the forward and reverse sequences of these 34 primers are provided in Table 2. A representative gel-based DNA fingerprint of maize landraces generated using SSR locus BNLG 1325 is provided in Figure 1. As is evident from Figure 1, the higher number of alleles per locus within landraces, as compared to hybrids and inbreds, is attributed to the genetic variation

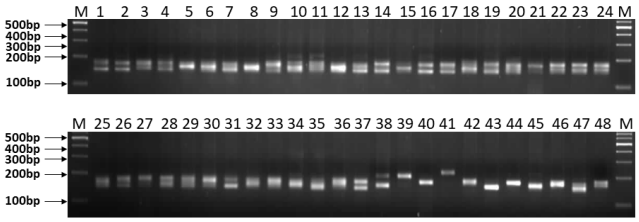

Figure 1: Representative DNA profile of maize (*Zea mays* L.) landraces generated using SSR locus BNLG 1325. Numbers (1-48) on top of the lanes denote the name of the landrace as depicted in Table 1. M is the 100 base pair molecular weight standard.

Table 3: Extent of polymorphism detected by SSR markers in maize (*Zea mays* L.) landraces

Primer	*TB*	*NP*	*GD*	*PIC*	*RP*	*EMR*	*MI*
BNL 128	3	3	0.532	0.439	1.71	3	1.60
BNL 238	4	4	0.502	0.495	1.36	4	2.01
BNL 240	2	2	0.269	0.233	0.64	2	0.54
BNL 1346	3	3	0.599	0.521	1.96	3	1.80
BNLG 105	2	2	0.489	0.369	1.70	2	0.98
BNLG 615	3	3	0.536	0.443	1.73	3	1.61
BNLG 1045	4	4	0.638	0.570	2.00	4	2.55
BNLG 1159	5	5	0.682	0.623	2.00	5	3.41
BNLG 490	5	5	0.534	0.496	1.40	5	2.67
BNLG 589	2	2	0.081	0.078	0.17	2	0.16
BNLG 1154	3	3	0.292	0.259	0.69	3	0.88
BNLG 1178	5	5	0.632	0.563	2.00	5	3.16
BNLG 1182	6	6	0.805	0.776	2.00	6	4.83
BNLG 1272	4	4	0.776	0.715	2.00	4	3.11
BNLG 1175	6	6	0.756	0.720	2.00	6	4.53
BNLG 1520	3	3	0.479	0.401	1.38	3	1.44
BNLG 1190	3	3	0.512	0.400	1.80	3	1.54
BNLG 1325	3	3	0.625	0.548	2.00	3	1.88
PHI 38920	2	2	0.249	0.218	0.58	2	0.50
PHI 116	2	2	0.464	0.356	1.46	2	0.93
PHI 119	4	4	0.548	0.446	2.00	4	2.19
UMC2134	2	2	0.371	0.302	0.98	2	0.74
UMC 2165	2	2	0.425	0.335	1.23	2	0.85
UMC 1068	3	3	0.371	0.323	0.93	3	1.11
UMC 1353	4	4	0.697	0.642	2.00	4	2.79
UMC 2017	2	2	0.487	0.368	1.68	2	0.97
UMC 1690	2	2	0.484	0.367	1.65	2	0.97
UMC 2043	3	3	0.534	0.417	2.15	3	1.60
UMC 2258	2	2	0.208	0.186	0.47	2	0.42
UMC 2325	2	2	0.488	0.369	1.68	2	0.98
Average	3.20	3.20	0.502	0.433	1.512	3.20	1.76

TB: total number of bands, NP: number of polymorphic bands, H: average heterozygosity, PIC: polymorphism information content, RP: resolving power; EMR: effective multiplex ratio, M: marker index

between individuals within an accession. Such multiple PCR amplification products can be ascribed to within-accession heterogeneity and segregation at a particular microsatellite locus (Dreisigacker *et al.*, 2005). High heterogeneity in landraces is an important adaptive trait under unfavorable environmental situations (Kyratzis *et al.*, 2019).

Various marker efficiency parameters for 30 polymorphic SSR primers such as total number of bands, polymorphic bands, gene diversity, PIC, resolving power, effective multiplex ratio and marker index were computed for diversity assessment and genetic relationships studies in maize landraces (Table 3). Total number of amplified bands varied from two for as many as ten primers to six for primers BNLG 1182 and BNLG 1175 with an average of 3.2 bands per primer. Dubey *et al.* (2009) also observed 3.2 mean number of alleles per locus in drought-tolerant and susceptible genotypes of maize. The mean number of bands (3.2) observed in our study is, however, low compared to other studies where mean number of bands was observed to vary from 3.8 to 11 (Sharma *et al.*, 2008; Arafayne *et al.*, 2018; Belalia *et al.*, 2019; Shyanowako *et al.*, 2018). This indicates that the allelic richness in the material in our study is comparatively low, which might be because the landraces in our study represent very limited geographical space of three districts of Jharkhand state of India. In addition, very high number of alleles in other studies could be the result of the inclusion of genetically very diverse material, which comprised landraces, open-pollinated varieties, inbreds, hybrids *etc*.

Gene diversity or expected heterozygosity, which is the probability that an individual is heterozygous for the locus in the population, varied from 0.208 (UMC 2258) to 0.805 (BNLG 1182) with an average value of 0.502. Tahir *et al.* (2016) observed the same extent of gene diversity (0.20-0.81) as did we. However, even higher levels of gene diversity have also been reported (Adu *et al.*, 2019). Polymorphism information content (PIC), a measure of polymorphism for a marker locus, ranged from 0.078 (BNLG 589) to 0.776 (BNLG 1182). The average PIC in the material was observed to be 0.433. More or less the same level of PIC has been reported earlier (Tahir *et al.*, 2016; Dubey *et al.*, 2009; Belalia *et al.* 2019). Resolving power is based on the distribution of alleles within the sampled genotypes and it correlates with the ability to distinguish analysed samples. The range of resolving power was from 0.17 (BNLG 589) to 2.15 (UMC 2043) with an average of 1.512 in the maize landraces evaluated in the present study. Effective multiplex ratio (EMR), the number of loci polymorphic in the material analysed for experiment fraction of polymorphic loci, ranged from 2 to 6. The product of EMR and gene diversity for polymorphic loci denoted as marker index was the highest for primer BNLG 1182 (4.83) and it was the lowest for primer BNLG 589 (0.16).

Genetic Diversity Among Maize Landraces

Average similarity, based on Jaccard's similarity coefficient, among 48 maize genotypes that included landraces, hybrids

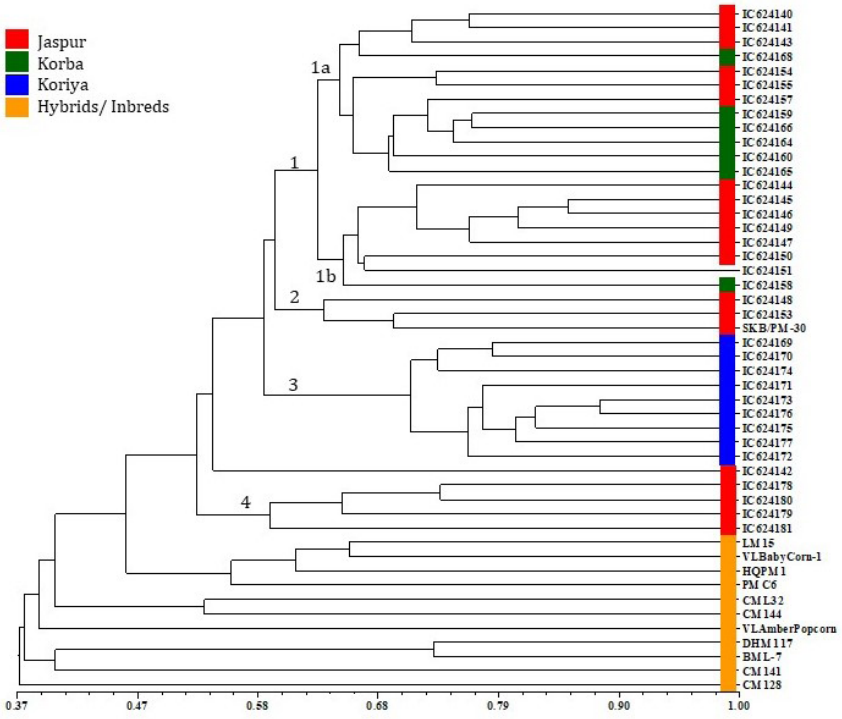

Figure 2: UPGMA based dendrogram generated using SSR markers depicting genetic relationships among maize (*Zea mays* L.) landraces and select hybrids and inbreds

Table 4: AMOVA analysis of maize landraces

Source of variation	*Degrees of freedom*	*Sum of squares*	*Variance components*	*Percentage of variation*
Among populations	2	67.10	2.29Va	18.33
Within populations	30	306.97	10.23Vb	81.67
Total	32	374.06	12.53	

Fst: 0.18331, Significance tests (10000 permutations) [Va and F_{ST} : P(rand. value > obs. value) = 0.00; P (rand. value = obs. value) = 0.000; p-value = 0.00 ± 0.00]

and inbreds, was found to be 0.475 with a minimum value of 0.122 (between landraces IC624173 and IC624176) and maximum value of 0.750 between landrace IC624178 and inbred CML32. Similarity among the 20 landraces from district Jaspur was found to be 0.401. A minimum similarity value of 0.149 (between landraces IC625145 and IC624146) and a maximum of 0.635 (between landraces IC624142 and IC624181) was observed in the landraces from Jaspur district. Among landraces from the district Korba, average similarity was observed to be 0.333 with a minimum similarity of 0.233 between the landraces IC624159 and IC624160 and a maximum similarity of 0.438 between the landraces IC624158 and IC624160. A minimum similarity of 0.122 between the landraces IC624173 and IC624176 and maximum similarity of 0.380 between the landraces IC624170 and IC624176 was observed among the landraces from district Koriya. However, the average similarity among the landraces from Koriya district was observed to be 0.251.

In earlier studies with maize landraces or inbred lines, Dubey *et al.* (2009) observed more or less similar genetic similarity (0.14–0.74) with an average of 0.31. Likewise, Saiyad and Kumar (2018) also reported the same level of genetic similarity between genotypes, ranging between 0.16 and 0.75 with an average of 0.49. A high level of genetic dissimilarity (72%) was observed in 124 landraces from Wuling mountain region in (Yao *et al.*, 2008) which might be true due to the diverse range of material in the study.

Population Structure and ANOVA Analysis

Cluster analysis of the SSR-based genetic similarity estimates provided substantial separation of maize landraces on the basis of their source district of the Jharkhand state. The UPGMA-based dendrogram (Figure 2) grouped all 37 maize landraces into four clusters. Hybrids and inbred lines included in the study were separated out from the landraces. Cluster 1 contained 20 maize landraces, all from Jaspur district or the Korba district of Jharkhand state. Although Jaspur and Korba landraces were intermixed in this cluster, within sub-cluster 1a Korba landraces, except IC624168 and IC624158 were having tendency to be grouped together. Sub-cluster 1b and cluster 4 contained all Jaspur landraces alone. All the maize landraces from Koriya district were grouped separately in cluster 3.

Principal coordinate analysis (PCoA) was undertaken in order to further substantiate the genetic relationship patterns observed using UPGMA clustering (Figure 3). The first three principal coordinates accounted for 26.7% of the total variation with first, second and third principal coordinates accounting for 11, 9.7 and 6.0% of the variability, respectively. Although very distinct patterns were not observed concerning the grouping of maize landraces, hybrids and inbreds (Figure 3A) were separately visible from the landraces (Figure 3B) in the three-dimensional plot. Among the maize landraces, four landraces IC624178, IC624179, IC624180 and IC624181 all from Jaspur district of Jharkhand tended to be differentiating from the rest (Figure 3C).

To further test these genetic relationships, a model-based clustering was done using the program STRUCTURE (Pritchard *et al.*, 2000). With no prior information about the populations and using an admixed model. STRUCTURE calculated that the estimate of the likelihood of the data (LnP(D)) was greatest when K=2 (Figure 4), suggesting that all maize genotypes in the study fell into one of the two clusters. The optimal K-value indicated that these two genetically distinct clusters primarily correspond to hybrids,

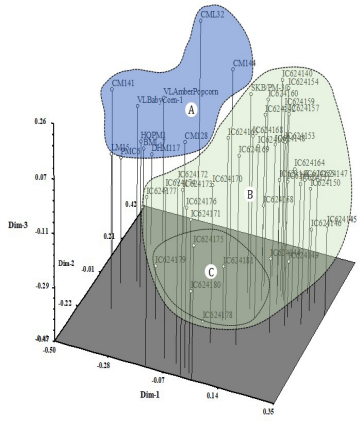

Figure 3: Principal coordinate analysis of maize (*Zea mays* L.) landraces generated using SSR markers

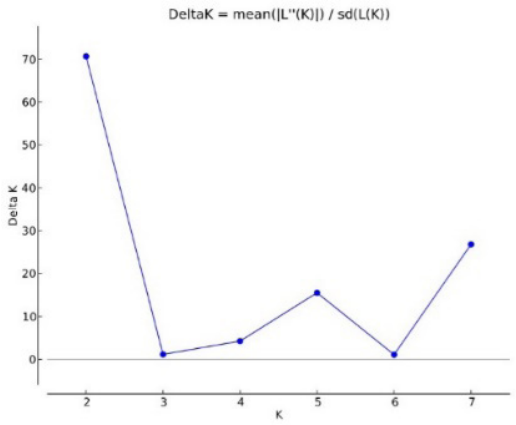

Figure 4: Delta K values for different groups assumed (K) in the structure analysis

inbred lines (Figure 5A), and landraces (Figure 5B). Among the group of landraces, four landraces IC624178, IC624179, IC624180 and IC624181 all from Jaspur district of Jharkhand tended to be differentiating from the rest as these were shown to be in the PCoA analysis as well.

Estimating the strength of the genetic population structure is usually done using F-statistics that decompose the genetic variance into within-population and among or (between) population components (Kempthorne and Wright, 1971). Fst, which quantifies the degree of population differentiation, depends on the balance among migration, mutation, and genetic drift. Analysis of Molecular Variance (AMOVA) performed using software Arlequin (Excoffier & Lischer, 2010) indicated that majority of genetic variation (81.67%) occurred within populations, while the variation between the populations (Fst) was 18.33% (Table 4). As we observed in our study, a similar level of population differentiation has been observed in Iranian maize genotypes using 18 SSR primers, revealing 17 and 83% of the total variation between and within populations, respectively (Tahir *et al.*, 2016). Higher levels of between population differentiation (24.4%) in maize landraces from northern Mexico (Herrera-Saucedo *et al.*, 2019) and in Algerian populations (22%) has been observed (Belalia *et al.*, 2019) as compared to our study which might be due to larger populations sizes in these studies.

DNA Fingerprinting for Identification of Maize Landraces

For the purpose of identification of maize landraces, discrimination power by which the efficiency of a primer alone or in combination with other primers can be tested was computed. The discrimination power, along with other parameters used to discriminate the maize landraces such as the probability of identity, confusion probability, and estimated discrimination power under the hypothesis when the sample size N tends to infinity and total number of undifferentiated landrace pairs are presented in Table 5. The probability of identity was found to be the lowest for primer BNLG 1272 (0.062) followed by for primers BNLG 1182 and BNLG 1175. However, this probability was the highest for primer BNLG 589 (0.847). The other two indices, namely, confusion probability and discrimination power, are interrelated. Subtracting confusion probability from unity gives discrimination power which was found to be the highest for primers BNLG 1182, BNLG 1175, UMC 1353, BNLG 1159 and BNLG 1272 in that order. Likewise, the estimated discrimination power was found to be highest in that order for these same five primers in the present study. More the number of undifferentiated landrace pairs by a primer, less efficient the primer is. These same five primers in the above order left 201, 257, 325, 342 and 362 pairs, respectively, and were more useful than the rest of the primers.

The expected number of indistinguishable pairs under the independence hypothesis are given in Table 6. From 48 genotypes in the study, 1128 pair-wise comparisons are possible. The theoretical total number of non-differentiating pairs with a particular primer is calculated by multiplying total pairs compared with the confusion probability of that particular primer. Out of 1128 pairs, primer BNLG 1182, selected based on its highest discrimination power, left 201 indistinguishable pairs. By adding primer BNLG 1175, with the second-highest discrimination power, the number of in-distinguishable pairs was reduced to 46. Similarly, in that order, the total number of indistinguishable pairs were reduced by adding primers UMC 1353, BNLG 1159 and BNLG 1272 in that order the total number of indistinguishable pairs were reduced to 13, four and one, respectively. Finally adding the primer BNLG 1045 all maize landrace pairs were distinguished. So, this set of six primers, namely, BNLG 1182, BNLG 1175, UMC 1353, BNLG 1159, BNLG 1272 and BNLG 1045 was found to discriminate all the landraces included in the study. Additional primers, if needed for discriminating larger sets of maize material, can be selected based on the discrimination power of the primers from Table 6.

Methods that allow varieties to be identified unequivocally are essential not only for variety identification but also to protect breeder and farmers rights. DNA markers have now reached a level of development that makes them suitable for this purpose, and when these are combined with conventional descriptors, the breeding stock can be unequivocally identified and any existing genetic diversity can be detected. As more and more closely related genotypes are being developed and need testing, the regular use of such markers is a fundamental requirement in the case of maize. DNA fingerprints of maize landraces can be generated, allowing easy identification

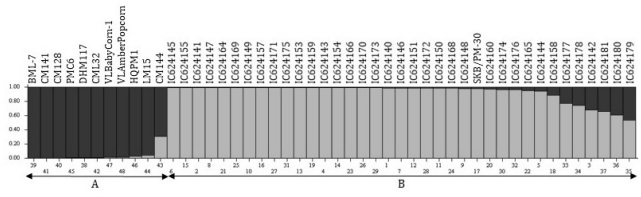

Figure 5: Model-based clustering of maize (*Zea mays* L.) landraces using STRUCTURE v2.3.4. Each landrace genotype is represented by a single row, which is partitioned into segments in proportion to the estimated membership in the two subpopulations. Numbers on the Y-axis show the subgroup membership and the X-axis shows the different genotypes as listed in Table 1

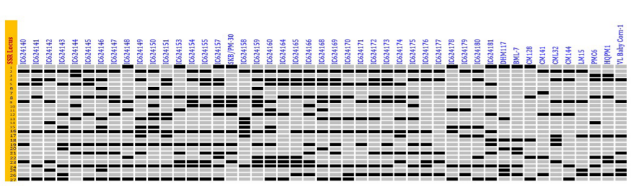

Figure 6: DNA fingerprints of maize (*Zea mays* L.) landraces represented as barcode generated using six most discriminating SSR primers (BNLG 1182, BNLG 1175, UMC 1353, BNLG 1159, BNLG 1272 and BNLG 1045)

Table 5: Discrimination indices among maize (*Zea mays* L.) landraces calculated using SSR markers

Primer	*PI*	*C*	*D*	*Order of D*	D_L	*X*
BNL 128	0.312	0.456	0.544	14	0.532	515
BNL 238	0.304	0.488	0.512	16	0.502	550
BNL 240	0.571	0.725	0.275	27	0.269	818
BNL 1346	0.239	0.388	0.612	9	0.599	438
BNLG 105	0.381	0.501	0.499	17	0.489	565
BNLG 615	0.309	0.453	0.547	12	0.536	511
BNLG 1045	0.199	0.348	0.652	6	0.638	393
BNLG 1159	0.161	0.303	0.697	4	0.682	342
BNLG 490	0.255	0.455	0.545	13	0.534	513
BNLG 589	0.847	0.917	0.083	30	0.081	1034
BNLG 1154	0.534	0.702	0.298	26	0.292	791
BNLG 1178	0.205	0.354	0.646	7	0.632	399
BNLG 1182	0.067	0.178	0.822	1	0.805	201
BNLG 1272	0.062	0.321	0.679	5	0.665	362
BNLG 1175	0.095	0.228	0.772	2	0.756	257
BNLG 1520	0.350	0.511	0.489	21	0.479	576
BNLG 1190	0.351	0.477	0.523	15	0.512	538
BNLG 1325	0.217	0.362	0.638	8	0.625	408
PHI 38920	0.595	0.746	0.254	28	0.249	841
PHI 116	0.395	0.527	0.473	22	0.464	594
PHI 119	0.306	0.440	0.560	10	0.548	497
UMC2134	0.465	0.621	0.379	25	0.371	701
UMC 2165	0.421	0.566	0.434	23	0.425	638
UMC 1068	0.444	0.621	0.379	24	0.371	700
UMC 1353	0.147	0.288	0.712	3	0.697	325
UMC 2017	0.382	0.503	0.497	19	0.487	567
UMC 1690	0.383	0.505	0.495	20	0.484	570
UMC 2043	0.334	0.453	0.547	11	0.534	511
UMC 2258	0.649	0.788	0.212	29	0.208	889
UMC 2325	0.381	0.502	0.498	18	0.488	566
Average	0.345	0.491	0.509		0.498	553.74

PI: probability of identity, C: confusion probability, D: discrimination power, D_L: Estimated discrimination power as N tends toward infinity, X: total number of undifferentiated pairs

with high precision. Using Microsoft Office Excel 2019, a DNA fingerprint barcode was constructed using 27 SSR allelic molecular weights (Figure 6). Six primers, namely, BNLG 1182, BNLG 1175, UMC 1353, BNLG 1159, BNLG 1272 and BNLG 1045 were identified for generating the DNA fingerprint barcode based on the high discrimination power of these markers. These SSR markers, therefore, can be used as alternative descriptors for landrace protection.

The study of genetic diversity in landraces is important in the light of narrowed genetic base owing to use of improved varieties and hybrids in maize. In addition to using modern tools to estimate diversity, traditional knowledge also needs to be exploited to augment the diversity patterns and optimize sampling of sub-samples in each particular landrace. Native edapho-climatic conditions to assess the variability also need consideration for agronomic evaluation of the landraces. *Ex-situ* conservation and strategies for characterization of within landrace variability needs to be developed. The analysis has led to the identification of specific, highly informative SSR primers, namely BNLG 1182, BNLG 1175, UMC 1353, BNLG 1159, BNLG 1272 and BNLG 1045, which significantly contributed in discrimination and diversity analysed in the study. Cluster analysis of molecular markers distinguished groups and identified landraces of maize that would be useful for the conservation and management of genebank collection and for possible utilization in maize breeding programs. SSR markers have a strong potential as a tool for complementary analysis of distinguishability, uniformity and stability required for cultivar registration and protection of plant breeders' rights. Strategies to retain diversity in landraces would include the development of core collections, allele mining and finding allelic variants for functional genes and genome-wide association studies (GWAS) for identifying marker-trait associations.

Table 6: Expected number of indistinguishable pairs under the independence hypothesis

Primer Combination	*Indistinguishable pairs per combination*
BNLG1182	201
BNLG1182 + BNLG1175	46
BNLG1182 + BNLG1175 + UMC1353	13
BNLG1182 + BNLG1175 + UMC1353 + BNLG1159	4
BNLG1182 + BNLG1175 + UMC1353 + BNLG1159 + BNLG1272	1
BNLG1182 + BNLG1175 + UMC1353 + BNLG1159 + BNLG1272 + BNLG1045	0
BNLG1182 + BNLG1175 + UMC1353 + BNLG1159 + BNLG1272 + BNLG1045 + BNLG1178	0
BNLG1182 + BNLG1175 + UMC1353 + BNLG1159 + BNLG1272 + BNLG1045 + BNLG1178 + BNLG 1325	0
BNLG 1182 + BNLG1175 + UMC1353 + BNLG1159 + BNLG1272 + BNLG1045 + BNLG1178 + BNLG1325 + BNL1346	0
BNLG1182 + BNLG1175 + UMC1353 + BNLG1159 + BNLG1272 + BNLG1045 + BNLG1178 + BNLG1325 + BNL1346 + PHI119	0

Acknowledgements

We greatly acknowledge financial support from the Indian Council of Agricultural Research (ICAR). Director, ICAR-NBPGR and Head, Division of Genomic Resources, ICAR-

NBPGR are also acknowledged for providing laboratory facilities for carrying out this work.

References

Abdurakhmonov, I. Y. (2016). *Introduction to microsatellites: basics, trends and highlights*. *https://doi.org/10.5772/66446*

Anderson, J. A., Churchill, G., Autrique, E., Tanksley, S., & Sorrells, M. (1993). Optimizing parental selection for genetic linkage maps. *Genome / National Research Council Canada = Génome / Conseil National de tRecherches Canada, 36, 181–186. https://doi.org/10.1139/g93-024*

Barbosa, A. M. M., Geraldi, I. O., Benchimol, L. L., Garcia, A. A. F., & Jr, C. L. S. (2003). Relationship of intra- and interpopulation tropical maize single cross hybrid performance and genetic distances computed from AFLP and SSR markers. *Euphytica*, *130*(1), 87–99. *https://doi.org/10.1023/a%3a1022381008721*

Belalia, N., Lupini, A., Djemel, A., Morsli, A., Mauceri, A., Lotti, C., Khelifi-Slaoui, M., Khelifi, L., & Sunseri, F. (2019). Analysis of Genetic Diversity and Population Structure in Saharan Maize (Zea Mays L.) Populations Using Phenotypic Traits and SSR Markers. *Genet. Resour. Crop Evol.*, **66(1):** 243–257. https://doi.org/10.1007/s10722-018-0709-3

Berhitu, E. C., Hiariej, A., & Seumahu, C. A. (2019). Genetic Characterization of Maize Kisar Var. Kuning Genjah and Maize Var. Bisi-II-Hibrida Based On Molekular RAPD Marker. *Agrotech. J.*, **4(2):** 91–98.

Boakyewaa Adu, G., Badu-Apraku, B., Akromah, R., Garcia-Oliveira, A. L., Awuku, F. J., & Gedil, M. (2019). Genetic diversity and population structure of early-maturing tropical maize inbred lines using SNP markers. *PLoS One*, **14(4):** e0214810–e0214810. *https://doi.org/10.1371/journal.pone.0214810*

Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.*, **32(3):** 314–331. *https://pubmed.ncbi.nlm.nih.gov/6247908*

Bracco, M., Cascales, J., Hernández, J. C., Poggio, L., Gottlieb, A. M., & Lia, V. V. (2016). Dissecting Maize Diversity in Lowland South America: Genetic Structure and Geographic Distribution Models. *BMC Plant Biol.*, *16*. *https://doi.org/10.1186/s12870-016-0874-5*

Dar, T. H., Shakeel, R., & Verma, S. (2018). Comparative Germplasm Characterization of Maize (*Zea mays* L.) in Rajouri Region of Pir Panjal Himalaya J & K (India), based on Morphological and ISSR Markers. *J. Crop Sci. Biotechnol.*, **21(1):** 43–55. *https://doi.org/10.1007/s12892-017-0128-0*

Excoffier, L, & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to performpopulation genetics analyses under Linux and Windows. *Mol. Ecol. Resour.*, **10:** 564–567.

Excoffier, Laurent, Smouse, P., & Quattro, J. (1992). Analysis of Molecular Variance Inferred From Metric Distances Among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data. *Genetics*, **131:** 479–491.

Gunjaca, J., Buhinicek, I., Jukic, M., Sarcevic, H., Vragolovic, A., Kozic, Z., Jambrovic, A., & Pejic, I. (2008). Discriminating maize inbred lines using molecular and DUS data. *Euphytica, 161 (1-2) Pp. 165-172, 2008*, *0014–2336*, *0014–2336*. *http://proxy-remote.galib.uga.edu:80/login?url=http://search.proquest.com/docview/43273210?accountid=14537*

Herrera-Saucedo, V., Córdova-Téllez, L., Santacruz-Varela, A., R, Y. M.-R., Rocandio-Rodríguez, M., & Á, C. H.-G. (2019). Genetic Diversity of Maize Landraces of Northern Mexico Analyzed through Microsatelites. *Agrociencia*, **53(4):** 535–548.

Hu, C., Zhang, L., Dong, Y., & Qiang. (2017). Revealing Core Heterotic Germplasm and Characterizing of Maize Inbred Lines Using SSRs. *Maydica*, *62*.

Kempthorne, O., & Wright, S. (1971). Evolution and the Genetics of Populations. Volume 2: The Theory of Gene Frequencies. *Biometrics*. *https://doi.org/10.2307/2528621*

Kyratzis, A. C., Nikoloudakis, N., & Katsiotis, A. (2019). Genetic variability in landraces populations and the risk to lose genetic variation. The example of landrace "Kyperounda" and its implications for ex situ conservation. *PLoS One*, **14(10):** e0224255–e0224255. *https://doi.org/10.1371/journal.pone.0224255*

Lahane, P. S., Khule, A. A., Jadhav, S. S., Parmar, S. K., Patil, M. B., Taware, S. D., & Ravidrababu, Y. (2016). Molecular characterizatio of maize landracs using random amplified polymorphic DNA markers. *Cell Tissue Res.*, **16(2):**

Lopes, M., El-Basyoni, I., Baenziger, P., Singh, S., Royo, C., Ozbek, K., Aktas, H., Özer, E., Ozdemir, F., Manickavelu, A., Ban, T., & Vikram, P. (2015). Exploiting genetic diversity from landraces in wheat breeding for adaptation to climate change. *J. Exp. Bot.*, **66.** *https://doi.org/10.1093/jxb/erv122*

Mahar, K. S., Agrawal, P. K., Babu, B. K., & Gupta, H. S. (2009). Assessment of genetic diversity among the elite maize (*Zea mays* L) genotypes adapted to North-Western Himalayan region of India using microsatellite markers. *J. Plant Biochem. Biotechnol.*, **18(2):** 217–220. *http://proxy-remote.galib.uga.edu:80/login?url=http://search.proquest.com/docview/43363999?accountid=14537*

Malik, N., Kumar, D., & Babu, B. K. (2020). Analysis of genetic divergence and population structure through microsatellite markers in normal and quality protein maize genotypes from NW Himalayan region of India. *VEGETOS*, **33(1):** 194–202. *https://doi.org/10.1007/s42535-020-00100-1*

Nguyen, T. Van, Doan, T. T. B., Leo, A. E., Bui, C. M., Taylor, P. W. J., & Ford, R. (2012). Application of Microsatellite Markers to Fingerprint and Determine the Representational Diversity within a Recently Established Elite Maize Inbred Line Breeding Program. *J. Agric. Sci.*, **4(6):** 258. *https://doi.org/10.5539/jas.v4n6p258*

Paetkau, D., Calvert, W., Stirling, I., & Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol.*, **4(3):** 347–354. *https://doi.org/10.1111/j.1365-294X.1995.tb00227.x*

Powell, W., Machray, G. C., & Provan, J. (1996). Polymorphism revealed by simple sequence repeats. *Trends Plant Sci.*, **1(7):** 215–222. *https://doi.org/https://doi.org/10.1016/1360-1385(96)86898-1*

Prevost, A., & Wilkinson, M. J. (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor. Appl. Genet.*, **98(1):** 107–112. *https://doi.org/10.1007/s001220051046*

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, **155(2):** 945. *http://www.genetics.org/content/155/2/945.abstract*

Punya, Sharma, V. K., Kumar, P., & Kumar, A. (2019). Agronomic Characters and Genomic Markers Based Assessment of Genetic Divergence and its Relation to Heterotic

Performance in Maize. *J. Environ. Biol.*, ***40*(5).**

Qi-Lun, Y., Ping, F., Ke-Cheng, K., & Guang-Tang, P. (2008). Genetic Diversity Based on SSR Markers in Maize (*Zea mays* L.) Landraces from Wuling Mountain Region in China. *J. Genet.*, **87(3):** 287–291. *https://doi.org/10.1007/s12041-008-0046-y*

Rohlf, F. J. (2004). *NTSYS-pc Numerical Taxonomy and Multivariate Analysis System, 2.11V. Exeter Software, Setauket, New York*.

Rosenberg, N. (2004). distruct: a program for the graphical display of population structure: PROGRAM NOTE. *Mol. Ecol. Notes*, **4:** 137–138. *https://doi.org/10.1046/j.1471-8286.2003.00566.x*

Roy, N. S., & Kim, N.-S. (2016). Genetic diversity analysis of maize lines using AFLP and TE-based molecular marker systems. *J. Genet. Genomics*, **38(10):** 1005–1012. *https://doi.org/10.1007/s13258-016-0461-z*

Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, R. A., & Allard, R. W. (1984). Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences (USA)*, **81:** 8014–8018.

Saiyad, M. M., & Kumar, S. (2018). Evaluation of Maize Genotypes for Fodder Quality Traits and Ssr Diversity. *J. Plant Biochem. Biotechnol.*, **27(1):** 78–89. *https://doi.org/10.1007/s13562-017-0418-6*

Singode, A., & Prasanna, B. M. (2010). Analysis of Genetic Diversity in the North Eastern Himalayan Maize Landraces Using Microsatellite Markers. *J. Plant Biochem. Biotechnol.*, **19(1):** 33–41. *https://doi.org/10.1007/bf03323433*

Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.*, **17(16):** 6463–6471. *https://doi.org/10.1093/nar/17.16.6463*

Tessier, C., David, J., This, P., Boursiquot, J. M., & Charrier, A. (1999). Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera* L. *Theor. Appl. Genet.*, **98(1):** 171–177. *https://doi.org/10.1007/s001220051054*

Tomkowiak, A., Bocianowski, J., Radzikowska, D., & Kowalczewski, P. Ł. (2019). Selection of Parental Material to Maximize Heterosis Using SNP and SilicoDarT Markers in Maize. *Plants (Basel, Switzerland)*, ***8*(9)**, 349. *https://doi.org/10.3390/plants8090349*

Wang, J., Zhong, G. Y., Chin, E. C. L., Register, J. C., Riley, R. D., Niebur, W. S., & Smith, J. S. C. (2002). Identification of parents of F1 hybrids through SSR profiling of maternal and hybrid tissue. *Euphytica*. *https://doi.org/10.1023/A:1015629600268*

Yao, Q., Fan, P., & Zou, S. (2008). Constructing a Core Collection for Maize (*Zea Mays* L.) Landrace from Wuling Mountain Region in China. *J. Integr. Agric.*, **7(12):** 1423–1432. *https://doi.org/10.1016/s1671-2927(08)60398-3*

Zehr, B. E., Dudley, J. W., Chojecki, J., Saghai Maroof, M. A., & Mowers, R. P. (1992). Use of RFLP markers to search for alleles in a maize population for improvement of an elite hybrid. *Theor. Appl. Genet.*, **83(6):** 903–911. *https://doi.org/10.1007/*

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2023.00036.2.04

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Microsatellite Markers based Identification of Zygotic and Nucellar Embryos in Polyembryonic Mango (*Mangifera indica* L.) Genotypes Vellaikulamban and Olour

Prashant Kalal[1*], P Nandeesha[2], Anuradha Sane[1], Reju M. Kurian[1], M Sankaran[1] and S. Sriram[3]

Abstract

The nucellar seedlings from polyembryonic mango seeds are useful as clonal rootstock while zygotic seedlings could be valuable for genetic variability. However, discriminating zygotic from nucellar seedlings originating from a single kernel based on morphology is impractical. The current study aimed to differentiate zygotic and nucellar embryos among multiple embryos in polyembryonic genotypes Vellaikulamban and Olour using nine SSR markers. Observed heterozygosity ranged from 0.182 (MiIIHR23) to 1.0 (MiIIHR17). LMMA12 had the maximum (0.823) polymorphic information content (PIC), and MiIIHR23 the minimum (0.562). Dendrogram using the neighbor-joining method demonstrated that embryos developed from nucellus grouped in the same cluster with the maternal parent, whereas zygotic embryos clustered in a distinctly different group. Six SSR markers identified the farthest embryo from the funicular point as zygotic in Olour, while in Vellaikulamban some discrepancy was observed for two markers.

Keywords: Polyembryony, Mango, Nucellar, Zygotic, Embryos, SSR, Vellaikulamban, Olour.

[1]Division of Fruit Crops, ICAR-IIHR, Bengaluru, Karnataka, India.

[2]Division of Basic Sciences, ICAR-IIHR, Bengaluru, Karnataka, India.

[3]Division of Crop Protection, ICAR-IIHR, Bengaluru, Karnataka, India.

***Author for correspondence:**

prashantskalal691@gmail.com

Received: 22/04/2022 **Revised:** 04/08/2022

Accepted: 14/11/2022

How to cite this article: Kalal, P., Nandeesha, P., Sane, A., Kurian, R.M., Sankaran, M., Sriram, S. (2023). Microsatellite Markers Based Identification of Zygotic and Nucellar Embryos in Polyembryonic Mango (*Mangifera indica* L.) Genotypes Vellaikulamban and Olour. *Indian J. Plant Genetic Resources*. 36(2), 227-232.

DOI: 10.5958/0976-1926.2023.00036.2.04

Introduction

Mango is a highly heterozygous and heterogeneous fruit crop that is propagated mainly by seed and grafting. Based on the kernel morphology, mango varieties are generally classified into two groups, namely monoembryonic and polyembryonic types. Monoembryonic mango varieties yield one seedling per seed which is expectedly zygotic in origin. Polyembryonic varieties often yield many seedlings. When multiple seedlings are produced, one of them is considered zygotic in origin, and the rest is from the maternal tissue (Viruel *et al*., 2005). Generally, the nucellar seedlings are used as rootstock for crop production whereas the weaker single zygotic seedling is discarded during propagation. The major challenges were faced during the selection of nucellar seedlings from zygotic ones by researchers and nurserymen. A plantlet origin was distinguished morphologically based on the seedling's vigorousness by many workers in the polyembryonic mango (Sachar and Chopra, 1957; Cordeiro *et al.,* 2006). The formation of all vigorous seedlings due to dominant nucellar growth over a weaker zygotic seedling was reported by Srivastava *et al.* (1988) in mango. Recently, some contradictory results related to the origin of multiple seedlings in the polyembryonic mango seeds were reported (Ochoa *et al*., 2012; Rocha *et al*., 2014) when molecular markers were employed. These include zygotic seedlings

originating first and being more vigorous in growth over following nucellar seedlings in the polyembryonic mango variety 'Uba' using ISSR markers by Rocha *et al*. (2014) and occurrence of varying percentages of zygotic embryos in the polyembryonic mango varieties by Ochoa *et al.* (2012) and Rocha *et al*. (2014). Differentiation of nucellar and zygotic seedlings through molecular markers was attempted *viz*., RAPD (Cordeiro *et al*., 2006; Srivastava *et al*., 2010; Ochoa *et al*., 2012), ISSR (Srivastava *et al*., 2010; Rocha *et al*., 2014), DAMD (Srivastava *et al*., 2010), SSR (Ruiz *et al*., 2000; Viruel *et al*., 2005; Sane *et al*., 2011; Kumar *et al*., 2020; Judith *et al*., 2021) but most of the results of these findings were not in agreement to each other in mango itself. With this background, the current study was planned to identify a polyembryonic kernel's zygotic and nucellar embryos using SSR markers.

Materials and Methods

DNA Isolation from Selected Genotypes

The open-pollinated ripened fruits were harvested from the trees of selected polyembryonic mango genotypes, namely Vellaikulamban and Olour during 2020-21. Thereafter, the seeds were extracted from fruits, and the hardy endocarp and tegmen from the kernel surface were removed. The kernels were washed using 70% alcohol and later washed with distilled water. The multiple segments (kernel embryos) of each polyembryonic kernel were separated individually and subsequently, each separated individual embryo of the kernel was considered as a sample, labeled sequentially to their position in the funiculus (1st, 2nd, 3rd, and 4th embryos were numbered from the funicular point in counter clockwise and then coded as A, B, C, and D) (Table 1) for DNA isolation. Usually, the polyembryonic mango kernel is compacted with more embryos (up to 10 in Olour). In the current study, polyembryonic kernels comprising up to 3 to 5 embryos were selected for ease of separating individual embryos and enabling the isolation of DNA from each embryo.

The matured leaves sample of selected mango mother trees of Vellaikulamban and Olour and their respective kernel embryo samples were collected for genomic DNA extraction using a CTAB (cetyltrimethylammonium bromide) based method described in Ravishankar *et al*., 2000. The quantity of extracted DNA was checked using a spectrophotometer (Gene Quanta, Amersham Biosciences, Holliston, USA) and the quality was checked through electrophoresis in 0.8% agarose gel.

PCR Conditions and Analysis

SSR markers were amplified using a fluorescence-based M13-tailed PCR reaction (Oetting, 1995) and the typical M13 tail was added to the SSR primers at the 5th position of the primer sequence (Schuelke, 2000). A total of nine SSR primers (Ravishankar *et al*., 2011) (Table 2) were used to genotype 22 mango samples (Table 1). The PCR was done in a 20 µL reaction volume comprising 2 µL of 20 ng DNA, Taq buffer 10X (2 µL, tris pH with 15 mM $MgCl_2$), dNTPs (1-µL of 10 mM), a forward primer of m-13 tail (1-µL of 5 pM), reverse primer (1-µL of 5 pM), 5 pM concentrated 1-µL fluorescent probes FAM, VIC, NED, and PET M13 primers, Taq polymerase (0.3 µL) and nuclease-free water (11.2 µL). A Bioer Life Pro Thermal Cycler was used to execute all of the PCR experiments (Bioer Technology, China). The PCR amplification condition was as follows: initial denaturation at 94°C for 1-minute, followed by 35 cycles of denaturation (94°C for 30 seconds), annealing (55°C for 30 seconds), and extension (72°C for 1-minute) steps, and a final extension at 72°C for 5 minutes.

On an automatic 96-capillary automated DNA sequencer (ABI 3730 DNA Analyzer, Applied Biosystems, USA), the amplified products labeled with FAM, VIC, PET, or NED were multiplexed and pooled before separation. To identify the exact fragment size of the PCR product, the data was further analyzed using the Peak Scanner software (Applied Biosystems, USA). The fragment size data were utilized to calculate the number of alleles per locus, observed

Table 1: Mango samples used in the study

Sample. No.	*Samples*	*Sample No.*	*Samples*
1	Vellaikolumban mother plant - VK-M	12	Olour mother plant - OL-M
2	Kernel-1 1st embryo (VK-1A)	13	Kernel -1 1st embryo (OL-1A)
3	Kernel -1 2nd embryo (VK-1B)	14	Kernel -1 2nd embryo (OL-1B)
4	Kernel -1 3rd embryo (VK-1C)	15	Kernel -1 3rd embryo (OL-1C)
5	Kernel -2 1st embryo (VK-2A)	16	Kernel -1 4th embryo (OL-1D)
6	Kernel -2 2nd embryo (VK-2B)	17	Kernel -1 5th embryo (OL-1E)
7	Kernel -2 3rd embryo (VK-2C)	18	Kernel -2 1st embryo (OL-2A)
8	Kernel -3 1st embryo (VK-3A)	19	Kernel -2 2nd embryo (OL-2B)
9	Kernel -3 2nd embryo (VK-3B)	20	Kernel -2 3rd embryo (OL-2C)
10	Kernel -3 3rd embryo (VK-3C)	21	Kernel -2 4th embryo (OL-2D)
11	Kernel -3 4th embryo (VK-3D)	22	Kernel -2 5th embryo (OL-2E)

Table 2: SSR primers used in the study.

Primers ID	*Primers (5'–3') sequence*
LMMA9	F- TTGCAACTGATAACAAATATAG
	R- TTCACATGACAGATATACACTT
LMMA10	F- TTCTTTAGACTAAGAGCACATT
	R- AGTTACAGATCTTCTCCAATT
LMMA12	F- AAAGATAGCATTTAATTAAGGA
	R- GTAAGTATCGCTGTTTGTTATT
MillHR29	F- CAACTTGGCAACATAGAC
	R- ATACAGGAATCCAGCTTC
MillHR17	F- GCTTGCTTCCAACTGAGACC
	R- GCAAAATGCTCGGAGAAGAC
MillHR18	F- TCTGACGTCACCTCCTTTCA
	R- ATACTCGTGCCTCGTCCTGT
MillHR23	F- TCTGACCCAACAAAGAACCA
	R- TCCTCCTCGTCCTCATCATC
MillHR-24	F- GCTCAACGAACCCAACTGAT
	R- TCCAGCATTCAATGAAGAAGTT
MillHR-26	F- GCGAAAGAGGAGAGTGCAAG
	R- TCTATAAGTGCCCCCTCACG

heterozygosity (Ho), expected heterozygosity (He), and polymorphic information content (PIC) value using Cervus 3.0 software, (Kalinowski *et al*., 2007). The results from the peak scanner were utilized to create barcodes. The barcodes were developed using a total of nine SSR markers and developed by Ravishankar *et al*. (2011) and Ravishankar *et al*. (2015) and Ravishankar *et al*. (2015). Short genetic sequences from a typical portion of the genome are used in barcoding. The 'Barcode of Life Database (BOLD, maintained by the University of Guelph) was used for all 20 mango samples. The neighbour-joining approach (NJ) was used to create a dendrogram for 22 mango samples using Darwin software (Perrier *et al*., 2003; http://darwin.cirad.fr/darwin).

Result and Discussion

Genetic Analysis of SSR Primers

The molecular analysis revealed that the selected SSR primers amplified alleles across all the samples and showed diverse levels of polymorphism. Among nine SSR markers, LMMA12 had a high amount of polymorphism (PIC = 0.823), while MilIHR23 had a low level of polymorphism (PIC = 0.562). The allele sizes and summary statistics of the SSR

Table 3 (a): Gene scan analysis of the Vellaikulamban mother plant and its progeny kernel embryos yielded PCR product sizes (bp) for nine SSR primers.

Primers ID	*Mother tree*	*Kernel-1*			*Kernel-2*			*Kernel-3*			
	VK-M	*VK-1A*	*VK-1B*	*VK-1C*	*VK-2A*	*VK-2B*	*VK-2C*	*VK-3A*	*VK-3B*	*VK-3C*	*VK-3D*
LMMA9	105/107	106/107	106/106	104/106	104/104	104/106	105/105	106/106	105/105	108/108	106/107
LMMA10	102/104	102/103	102/105	104/105	103/104	103/104	102/105	104/105	105/105	102/105	102/105
LMMA12	104/105	102/105	102/105	105/106	104/105	103/105	103/105	105/106	104/105	103/105	105/105
MillHR29	109/109	109/109	109/109	108/109	108/109	109/109	109/109	109/109	109/110	109/109	109/109
MillHR17	108/109	108/109	108/109	108/109	108/109	108/109	108/110	109/110	109/110	108/109	109/110
MillHR18	108/109	109/109	109/111	109/109	108/108	107/109	109/109	107/109	108/108	102/104	108/110
MillHR23	105/106	106/106	105/105	105/105	104/105	105/105	105/105	104/104	104/104	105/105	105/105
MillHR24	105/107	105/107	108/108	108/108	105/106	105/108	105/108	108/108	108/110	108/110	108/108
MillHR26	109/111	109/09	110/111	109/110	110/110	106/107	105/105	110/110	109/110	108/110	104/105

Table 3 (b): Gene scan analysis of the Olour mother plant and its progeny kernel embryos yielded PCR product sizes (bp) for nine SSR primers.

Primers ID	*Mother tree*	*Kernel-1*					*Kernel-2*				
	OL-M	*OL-1A*	*OL-1B*	*OL-1C*	*OL-1D*	*OL-1E*	*OL-2A*	*OL-2B*	*OL-2C*	*OL-2D*	*OL-2E*
LMMA9	104/106	106/106	106/106	104/105	104/104	114/115	105/105	106/106	105/106	105/105	105/107
LMMA10	108/109	107/108	108/108	108/108	107/109	117/119	107/107	108/110	108/108	108/108	108/108
LMMA12	109/111	109/110	109/110	109/112	109/109	115/117	109/110	110/112	110/110	110/111	109/110
MillHR29	104/106	104/104	105/105	105/106	106/106	120/120	105/106	105/105	106/107	105/105	104/106
MillHR17	108/110	108/109	109/110	108/109	108/109	108/110	108/109	108/109	109/110	107/109	109/110
MillHR18	108/109	108/108	107/109	110/110	107/109	107/109	107/107	110/110	107/107	106/107	106/108
MillHR23	106/106	105/105	105/105	104/106	105/105	110/112	105/105	104/104	104/104	106/106	105/105
MillHR24	105/107	106/108	105/107	107/107	106/108	108/110	105/107	108/108	108/108	105/108	106/106
MillHR26	109/110	109/109	110/110	109/109	109/109	113/113	109/112	109/110	111/111	108/109	109/110

Table 4: List of SSR primers and their characteristics based on genetic analysis of polyembryonic kernel embryos of Vellaikolumban and Olour along with the mother plants.

Primer ID	*Number of alleles per locus (k)*	*Observed heterozygosity (Ho)*	*Expected heterozygosity (He)*	*Polymorphic information content (PIC)*
LMMA9	7	0.455	0.766	0.710
LMMA10	10	0.682	0.851	0.813
LMMA12	11	0.864	0.86	0.823
MilIHR29	8	0.364	0.759	0.712
MilIHR17	4	1	0.67	0.586
MilIHR18	8	0.545	0.797	0.746
MilIHR23	5	0.182	0.631	0.562
MilIHR24	5	0.636	0.725	0.669
MilIHR26	10	0.5	0.807	0.761
Mean	7.5	0.581	0.762	0.709
Range	4 to 11	0.182 to 1.00	0.631 to 0.851	0.562 to 0.823

markers are given in Tables 3(a), (b), and 4. At nine SSR loci, at least an allele of the maternal parent was found in most of the seed embryos except for a few embryos that lack a maternal allele. This validates the genetic composition of embryos that were similar to the mother parent and those that were not, which was the major objective of this study. Microsatellite markers are the most reliable and robust because of their co-dominant, multiallelic nature and as well as amenable to multiplexing (Powel *et al*., 1996; Collard *et al*., 2005). The gene scan analysis of 22 samples using 9 SSR markers detected an average of 7.5 alleles per locus, ranging from 4 alleles in MilIHR17 to 11 alleles in LMMA12. Ravishankar *et al*. (2015) found 23.37 mean alleles per locus (K), while Ravishankar *et al*. (2017) found 16.6 alleles per locus and concluded that a genotype's heterozygosity determines the number of alleles per locus. Recently, similar work done by Sajana *et al*. (2021) used 16 SSR markers for 6 polyembryonic mango samples and achieved similar results of 9.25 alleles per locus. The expected heterozygosity ranged from 0.631 in MilIHR23 to 0.851 in LMMA10. The observed heterozygosity ranged from 0.182 in MilIHR23 to 1.00 in MilIHR-17 indicating high polymorphism. The PIC value was maximum (0.823) for LMMA12 and minimum (0.562) for the MilIHR23 marker. All of the primers used in this study were found to be highly informative, reproducible, and polymorphic in distinguishing the maternal and zygotic embryos (Eiadthong *et al*., 1999).

Prediction of Embryo Origin based on Allelic Similarity

Nine SSR markers were used to identify the origin of the embryos based on the allelic size of parental plants of polyembryonic genotypes with their progeny kernel embryos. With the comparison of the maternal parent in both polyembryonic cultivars Vellaikulamban and Olour, most of the embryos were observed as a nucellar origin, while a few polyembryony seeds produced one zygotic embryo per kernel (VK-2B, VK-2C, VK-3C, VK-3D, and OL-1E) with different SSR profiles [Table 3 (a, b) and 5]. The current results of the molecular study confirm the findings of Kumar *et al*. (2020) who reported the frequency of one zygotic seedling per polyembryonic mango seed. When compared to the Vellaikulamban mother plant, VK-2B and VK-2C embryos were identified as zygotic embryos by the MilIHR26 marker, while VK-3C (3rdembryo of kernel no. 3) was indicated as the zygotic by MilIHR18 marker and another embryo VK-3D was also identified as a zygotic embryo through MilIHR26 marker; however, the rest of the Vellaikulamban embryos were identified as nucellar origin based on the SSR profile (Table 3a and 5). When compared to the Olour mother plant, the embryo OL-1E (5th embryo of kernel no.1) was identified as the zygotic origin with the six markers LMMA9, LMMA10, LMMA12, MilIHR29, MilIHR23, and MilIHR26 (Table 3b and 5). All markers confirmed that all the embryos of Olour second kernel (OL-2A, OL-2B, OL-2C, OL-2D, and OL-2E) were nucellar (Table 5).

Distance Matrices

The dendrogram created using the neighbour joining approach revealed three major clusters among 22 samples (Figure 1). Cluster I contain two subclusters in which, the embryos such as OL-1A, OL-1D, OL-2A, and OL-2E were found closely related and genetically similar to OL-M. The second subcluster of cluster I includes two sub-subclusters in which embryos *viz* OL-2B, OL-1C, OL-2C, and OL-2D were found closely related but distantly related to the OL-1B embryo. Cluster II contains two subclusters: the Vellaikulamban mother plant and their genetically identical nucellar embryos. The first subcluster of cluster II contains embryos viz VK-3D, VK-1B, VK-3C, VK-2C, VK-3B, VK-3A, VK-2B, VK-1C, VK-2A. The second subcluster included the VK mother plant along with the embryo VK-1A. Overall, cluster II revealed that most markers (excluding MilIHR26 and MilIHR18) could identify nucellar embryos similar to the Vellaikulamban

Table 5: SSR primers differentiated the zygotic and nucellar origin of polyembryonic kernel embryos of Vellaikulamban and Olour [Table 5. constructed using gene scan analysis data from Table 3(a) and 3(b)]

Primers ID	M-tree	Kernel-1			Kernel-2			Kernel-3				M-tree	Kernel-1				Kernel-2					
	VK-M	VK-1A	VK-1B	VK-1C	VK-2A	VK-2B	VK-2C	VK-3A	VK-3B	VK-3C	VK-3D	OL-M	OL-1A	OL-1B	OL-1C	OL-1D	OL-1E	OL-2A	OL-2B	OL-2C	OL-2D	OL-2E
LMMA9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Z	N	N	N	N	N
LMMA10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Z	N	N	N	N	N
LMMA12	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Z	N	N	N	N	N
MilIHR29	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Z	N	N	N	N	N
MilIHR17	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MilIHR18	N	N	N	N	N	N	N	N	N	Z	N	N	N	N	N	N	N	N	N	N	N	N
MilIHR23	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Z	N	N	N	N	N
MilIHR24	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MilIHR26	N	N	N	N	N	Z	Z	N	N	N	Z	N	N	N	N	N	Z	N	N	N	N	N

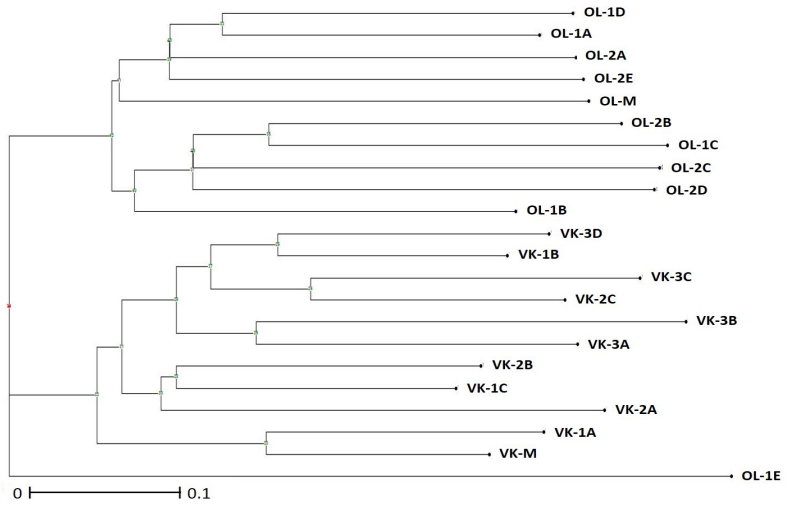

Figure 1: Dendrogram of zygotic and nucellar-originated embryos of polyembryonic genotypes along with mother plants based on SSR markers. VK-M Vellaikulamban mother plant, OL-M – Olour mother plant.

mother plant. In cluster III, the only embryo OL-1E was observed as a zygotic origin however this embryo was found distinct from its maternal Olour parent. Clusters I and II contained true-to-type nucellar embryos identical to their polyembryonic mother plants; however, a zygotic embryo (OL-1E) of polyembryonic Olour kernel in cluster III displayed a genetic distinctiveness.

In the present investigations, the embryos of polyembryonic Vellaikulamban and Olour kernels produce a zygotic embryo, or sometimes it may degenerate due to the dominance of the nucellar embryo's growth. According to the present findings, all individual Vellaikulamban kernel embryos had exhibited maternal origin except a few embryos. In Vellaikulamban, out of three kernels, only the first kernel had all embryos that resembled the maternal parent. In the second kernel, the 2nd and 3rd embryos were identified as zygotic by single primer MilIHR-26. In the third kernel, 3rd and 4th embryos were found to be zygotic by two primers, namely MilIHR18 and MilIHR26. In Olour, out of two kernels, the second kernel had embryos that resembled the maternal parent and in the first kernel, the 5th embryo was zygotic as shown by 6 primers. The identified primers, namely LMMA9, LMMA10, LMMA12, MilIHR29, MilIHR23, and MilIHR26 can be used for differentiating nucellar and zygotic embryos in Olour polyembryonic genotype. An attempt has been made for the first time to use individual kernel segments as samples for the identification of zygotic and nucellar embryos to overcome the ambiguity during the selection of seedlings of the selected polyembryonic genotypes. In Olour all six markers uniformly identified the embryo located farthest from the funicular point as zygotic when it was present. However, all the markers were not equally effective in discriminating zygotic and nucellar origin in the case of Vellaikulamban.

Acknowledgment

The author is thankful to ICAR-IARI, New Delhi, and ICAR-IIHR, Bengaluru for financial help under IARI Fellowship

and University Grant Commission for Rajiv Gandhi National Fellowship during the doctoral degree of the corresponding author.

References

Collard, B. C., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica*, **142:** 169-196.

Cordeiro, M. C. R., Pinto, A. C. Q., Ramos, V. H. V., Faleiro, F. G., & Fraga, L. M. S. (2006). Identificaçao da origem genética de plantulas em sementes poliembrionicas de mangueira (*Mangifera indica*, L.) cv. Rosinha por meio de marcadores RAPD. *Rev. Bras. Frutic.* **28(3):** 454-457.

Eiadthong, W., Yonemori, K., Sugiura, A., Utsunomiya, N., & Subhadrabandhu, S. (1999). Characterization of 15 microsatellite loci from mango (*Mangifera indica* L.) and cross-species amplification in closely related taxa. *Molecular Ecology Notes*, **5:** 625-627.

Judith, D. L. C. M., Monter, A. V., Mancera, H. A. Z., & Izquierdo, S. I. C. (2021). Identification of zygotic and nucellar seedlings, originated by the largest embryo in mango seeds cv Ataulfo, using simple-sequence repeat (SSR). *Afr. J. Agric. Res.*, **17(5):** 794-801.

Kumar, K., Srivastav, M., Singh, S. K., & Singh, A. (2020). SSR marker-based differentiation of zygotic and nucellar seedlings in mango (*Mangifera indica*). *Indian J. Agric. Sci.,* **90(11):** 2101–7.

Ochoa, E. D. C. M., Andrade-Rodriguez, M., Rodríguez, M. R., & Monter, A. V. (2012). Identification of zygotic and nucellar seedlings in polyembryonic mango cultivars. *Pesqui. Agropecu. Bras.*, **47:** 1629-1636.

Ravishankar, K. V., Anand, L., & Dinesh, M. R. (2000). Assessment of genetic relatedness among mango cultivars of India using RAPD markers. *J. Hortic. Sci. Biotechnol.*, **75(2):** 198-201.

Ravishankar, K. V., Bommisetty, P., Bajpai, A., Srivastava, N., Mani, B. H., Vasugi, C., Rajan, S., & Dinesh, M. R. (2015). Genetic diversity and population structure analysis of mango (*Mangifera indica*) cultivars assessed by microsatellite markers. *Trees*. **29(3):** 775-83.

Ravishankar, K. V., Mani, B. H. R., Anand, L., & Dinesh, M. R. (2011). Development of new microsatellite markers from Mango (*Mangifera indica*) and cross-species amplification. *Am. J. Bot.*, **98(4):** e96-e99.

Rocha, A., Salomao, T. M. F., Siqueira, D. L. D., Cruz, C. D., & Salomao, L. C. C. (2014). Identification of 'Uba' mango tree zygotic and nucellar seedlings using ISSR markers. *Revista Ceres*, **61:** 597-604.

Ruiz, C., Paz Breto, M., & Asins, M. J. (2000). A quick methodology to identify sexual seedlings in citrus breeding programs using SSR markers. *Euphytica*, **112:** 89-94.

Sachar, R. C., & Chopra, R. N. (1957). A study of the endosperm and embryo in *Mangifera* L. *Indian J. Agric. Sci.*, **27(2):** 219-228.

Sajana, S., Thomas, P., Ravishankar, K. V., Nandeesha, P., Kurian, R. M., Sane, A., & Bindu, H. (2021). Marker-assisted confirmation of maternal or zygotic origin of embryogenic cultures and regenerated plantlets of two polyembryonic mango cultivars using SSR markers. *J. Hortic. Sci. Biotechnol.*, **96(2):** 201-208.

Sane, A., Dinesh, M. R., Ravishankar, K. V., Ravishankar, H., & Vasugi, C. (2011). Implications of polyembryony on the growth performance in mango cultivars. In *Global Conference on Augmenting Production and Utilization of Mango: Biotic and Abiotic Stresses,* **1066:** 47-54.

Srivastava, K. C., Rajput, M. S., Singh, N. P., & Lal, B. (1989). Rootstock studies in mango cv. Dashehari. *Acta Hortic.,* **231:** 216-219.

Srivastava, N., Bajpai, A., Chandra, R., Rajan, S., Srivastava, M. K., & Muthukumar, M. (2010). Parentage and hybridity confirmation in mango hybrids by three DNA marker system combinations. *J. Biotechnol.*, **(150):** 492.

Viruel, M. A., Escribano, P., Barbieri, M., Ferri, M., & Hormaza, J. I. (2005). Fingerprinting, embryo type and geographic differentiation in mango (*Mangifera indica* L., Anacardiaceae) with microsatellites. *Mol. Plant Breed.*, **15:** 383-393.

Wayne, P., Gordon, C. M., & Jim, P. (1996). Polymorphism revealed by simple sequence repeats. *Trends Plant Sci.*, **1(7):** 215-222.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2022.00036.2.05

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Eco-geographic Survey, *In-situ* Characterization and Spatial Analysis of Phenotypic Traits Diversity among the Maize Landraces Collected from Chhattisgarh

Santosh K. Bishnoi[1], Pavan K. Malav[2]*, Kartar Singh[3], Rahul M. Phuke[4], S.B. Choudhary[5], Hariom K. Sharma[6] and S.P. Ahlawat[2]

Abstract

Exploration and germplasm collection expedition undertaken in the three districts of Chhattisgarh state in the present study resulted in the collection of 43 diverse local maize genotypes. The collected accessions were characterized *in-situ* on a farmer's field for six ear and seed-specific quantitative characters and the recorded data was analyzed for quantification and analysis of the genetic variability among the accessions. Significant variability was recorded in all the studied traits *viz*. ear length (7.33–26.67 cm), ear girth (8.00–16.33 cm), number of kernel rows per ear (8-18), number of kernels per ear row (12.67–45.33), ear weight (25.45–204.88 g) and 100 kernel weight (9.74–38.66 g). The first three components explained 99.61% of the total variation; the first principal component represented 97.33%. Cluster analysis based on Euclidean distances classified 43 maize genotypes into five clusters with clustering together of 9, 18, 13, 2 and 1 accessions. Geo-referencing analysis of recorded data was done using DIVA-GIS software. The diversity pattern of maize germplasm accessions revealed by the grid maps confirmed the existence of considerable genetic diversity for all the six quantitative traits in the surveyed area also discussed in this study.

Keywords: *Zea mays*, Germplasm, *In-situ* characterization, Diversity analysis, DIVA-GIS.

[1]ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana, India,

[2]ICAR-National Bureau of Plant Genetic Resources, New Delhi, Delhi, India,

[3]ICAR-National Bureau of Plant Genetic Resources, Jodhpur, Rajasthan, India,

[4]ICAR-Indian Agricultural Research Institute, Indore, Madhya Pradesh, India,

[5]ICARNational Bureau of Plant Genetic Resources, Regional Station, Ranchi, Jharkhand, India,

[6]ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan, India

***Author for correspondence:**

pavanmalav1988@gmail.com

Received: 28/04/2022 **Revised:** 26/04/2023

Accepted: 26/04/2023

How to cite this article: Bishnoi, S.K., Malav, P.K., Singh, K., Phuke, R.M., houdhary, S.B., Sharma, H.K. and Ahlawat, S.P. (2023). Eco-geographic Survey, In-situ Characterization and Spatial Analysis of Phenotypic Traits Diversity among the Maize Landraces Collected from Chhattisgarh. *Indian J. Plant Genetic Resources*. 36(2), 233-242. **DOI:** 10.5958/0976-1926.2022.00036.2.05

Introduction

The breeding progress in maize is dependent on the extent of genetic diversity in its gene pool, most importantly the landraces (Delucchi *et al.,* 2012; Azeez *et al.,* 2018). Therefore, the collection and *ex-situ* conservation of the landraces is a pre-requisite for appreciating their importance as genetic resources and for the development of complementary conservation methodologies (Vilaro *et al.,* 2020). There is a lacking of agronomic and genetic data for the collected landraces limiting their use and management (Azeez *et al.,* 2018). Modern commercial agricultural practices involving homogeneous high-yielding cultivars have resulted in the decline of landrace diversity fueled by global climate change (Mir *et al.,* 2020). The landrace diversity should not only be conserved from a gene source point of view but also from the point of view of sustainable agriculture because of their resiliency/adaptation towards abiotic and biotic stresses prevalent in a particular geography and that they have a preferred taste and sensory properties (Tokatlidis and Vlachostergios, 2016; Dwivedi *et al.,* 2016; Casanas *et al.,* 2017).

The eco-geographic data associated with the collected germplasm accessions has proved extremely useful for trait identification in different crops, including maize (Vilaro *et al.,* 2020). A germplasm accession accompanied with complete passport

information and preliminary statistics about key ear and kernel traits is of immense value to the breeders for starting of a pre-breeding programme.

The maize germplasm from India represents a very important component in the world's maize collections. The role of farmer conservators has been well recognized in India in the Protection of Plant Varieties and Farmers' Rights Act, 2001 (PPVF&RA, 2001) and the Biological Diversity Act, 2002 (BDA, 2002).

Indian maize landraces are a very important source of single cross maize hybrids (SCHs). The maize germplasm diversity having primitive characters of popcorns and prolificacy from North-Eastern and North-Western Hill regions of the country have been substantially collected and conserved, but less emphasis has been laid on the maize germplasm from other parts (Prasanna 2010, 2012; Yadav *et al.,* 2015 and Dar *et al.,* 2018).

A total of 11699 maize accessions are conserved in the Indian National Gene Bank til now. Out of these only 275 accessions are conserved from Chhattisgarh state and 43 accessions are targeted for the present study. Important gaps were reported in the collections from Chhattisgarh (Pandey *et al.,* 2015); *http://www.nbpgr.ernet.in/PGR_Databases.aspx)*, which needed to be filled for building of a comprehensive national collection of maize germplasm. Further, the state of Chhattisgarh is the target for the popularization of SCHs. However, a low hybrid adoption rate of less than 30% was reported (Yadav *et al.,* 2015) which presented a unique opportunity to collect the indigenous maize diversity from the previously unexplored areas of the state.

Therefore, the present study was undertaken with an objective of filling the maize germplasm gaps by undertaking an eco-geographic survey, collection and *in-situ* characterization mission from the three ethnically and eco-geographically diverse districts of Chhattisgarh *viz*. Jaspur, Koriya and Korba.

Material and Methods

Area Prioritization

In the present study, three districts *viz*. Jaspur, Koriya and Korba located in the North-Eastern part of the Chhattisgarh state were targeted for execution of the eco-geographic survey and germplasm collection mission. The geographic area explored was prioritized based on the gaps in the maize collections of the National Gene Bank. The exploration and germplasm collection mission was included in the National Exploration Plan of the ICAR-National Bureau of Plant Genetic Resources, New Delhi for 2017. The prioritized area represents previously unexplored, predominantly tribal with primitive agriculture and highly inclined for hybrid maize adoption with high probability of the erosion of maize genetic diversity.

The Eco-geographic Survey

As suggested by Brown and Marshall (1995), the modalities of the eco-geographic survey were worked out beforehand, including the itinerary to be undertaken and the establishment of prior contacts with the local Krishi Vigyan Kendras which are the governmental grassroots level extension institutions, to help locating the potential sites of maize germplasm diversity in the targeted areas. The total time of survey was divided based on the prior information about the presence of diversity received from the local collaborators. The exploration and germplasm collection mission was executed from 21–30th September 2017. The time of the survey and collection mission was so adjusted to coincide with the physiological maturity of the maize crop in most of the targeted areas. The starting point and the direction of the transacts were randomized at the time of preparation of the itinerary and minimum distance among the sampling points were employed as per Brown and Marshall (1995).

Germplasm Sampling

The targeted area, with particular focus on deep-seated interior germplasm diversity pockets, was transacted as per the pre-planned itinerary with 100 to 150 km (Singh and Srivastava, 2004) per day and germplasm collection was attempted as and when sighted. Large samples were particularly attempted in case of highly heterogeneous populations (Brown and Briggs, 1991). Random sampling consisting of a magnitude of different genotypes as per Brown and Marshall (1995) to ensure maximum "allelic richness" which is the direct measure of the value of an accession. The ripe cobs (ears) were collected from the farmer's field, threshing yard and/or from the farm stores employing the bulk random sampling and the frequency of sampling (number of samples per site) was adjusted based on "on-the-spot" observations on the variability available. Each collected maize germplasm accession was assigned a specific collector number and status of the population as a landrace/non-improved cultivar was confirmed by interpersonal interactions with the concerned farmers and the household elderlies.

In-situ Data Recording

The germplasm collection and subsequent characterization are often not in a continuum under real conditions and the beginning of characterization may take an unexpectedly long time due to many logistic and other constraints. *In-situ* germplasm characterization on reproductive/commercial plant parts (ear and kernels in case of maize) makes the preliminary identification of trait-specific germplasm easy as it gives a broad insight about the genetic worth of the collected material based on the observations recorded during collection. The *in-situ* data recording involved on the spot recording of two different kinds of data, the passport data and the quantitative data on ear/seed-specific traits.

The information under the passport data was recorded in the "standard passport data book" of the ICAR-NBPGR and involved assigning of a specific collector number to the collected accession, vernacular name of the species, name of the landrace, biological status (landrace/variety/population), type of material (kernels/ear), date of collection, collecting site/acquisition source (farmer's field/farm store/threshing yard), frequency of occurrence, sample type (population/individual), sampling method (random/specific), habitat (cultivates/wild), site of the collection (Basti (locality)/Village/Developmental block/District/State) and the latitude, longitude and altitude of the collection site using a hand-held Garmin GPS device. All the essential fields of the passport data book were duly filled on the spot. The ears were shelled and seeds were mixed after recording the data. The data on six ear and kernel-specific traits *viz*. ear length, ear girth, number of kernel rows per ear, number of kernels per ear-row, ear weight and 100 kernel weight were recorded on three randomly selected ears. The kernel color, kernel-row arrangement and kernel type (flint/dent/pop) was recorded on a visual basis.

Data Analysis

The public domain software DIVA-GIS version 7.5 (Hijmans *et al.*, 2012) freely available on *www.diva-gis.org* was used for spatial analysis and geo-referencing of the collected maize germplasm diversity by generation of grid maps displaying presence points of the trait specific maize germplasm in a specific geographic area along with the map showing vegetation index i.e. intensity of vegetation in the collected area representative of cropping intensity. The coordinates of the collection were recorded in decimals and used for geo-mapping of maize germplasm accessions. Trait-wise geo-referencing (raster cell size=20 minute) was done using statistics option of points to grid tab of the analysis option of DIVA GIS software. The data recorded on six quantitative traits was analyzed for descriptive statistics, principle component analysis using PAST software (Hammer *et al*., 2001). Cluster analysis based on quantitative traits was done using NTSYSpc v. 2.20 (Rohlf, 2005). For cluster analysis first estimates of Euclidean distance coefficients were carried out for all pairs of genotypes using SIMINT module of NTSYS software. The resulted matrices of Euclidean dissimilarity coefficient were applied to the SAHN programme of NTSYS software to construct UPGMA dendrogram. Simple correlation coefficients between all pairs of quantitative traits were obtained using mean values (Steel and Torrie, 1980).

Results

The Eco-geographic Survey, Germplasm Collection and Passport Data

During the present eco-geographic survey and collection expedition, a total of 43 maize accessions were collected from a geographic area spanning three districts *viz*. Jaspur, Koriya and Korba of the Chhattisgarh state. The collected germplasm accessions varied primarily in morpho-phenology, spatial origin, eco-geography of the collection site and anthropo-ethnic backdrop. During the survey, the maize germplasm was collected from the farmer's field (32), threshing yard (8) and farm store (3) with the number of accessions acquired from each source given in the parenthesis. The number of accessions acquired from each source signifies the relative maturity duration as late maturing accessions were still in the farmer's field, medium maturity accessions were those collected from the threshing yard and early to very early maturing accessions already harvested, threshed and stored in the farm store, therefore collected from there. It is evident that most of the germplasm accessions collected during the present study fall into the category of late maturing types. The altitudinal range of the collected material ranged from 312 meters from mean sea level to 1089 meters from the mean sea level (Table 1). This range was classified into different categories of altitude of 100 MSL. By this categorization, a total of 16 accessions were collected between the altitudinal ranges of 451–550 MSL. The altitudinal range of 851–1050 MSL was found to be devoid of maize cultivation in the area explored under the present study. The passport details of all the collected accessions along with the geographic coordinates of the collection, are presented in Table 1.

In-situ Phenotypic Characterization

Significant variation in kernel color, ear shape, ear size, kernel type, row arrangement, ear length, number of kernel rows per ear, number of kernels per ear, number of kernels per row and 100 kernel weight was observed and collected. The collected accessions in the present study were classified into categories based on the differences in the kernel colors *viz*. yellow, yellow and white, yellow with white tinge, pale, white, etc. (Table 1). However, the base color of most of the collected accessions was yellow or the variants thereof, signifying the local farmer's preference for yellow or related colors. The accessions collected in the present study (Figure 1) exhibited a particular pattern regarding their ear shape. The ears were

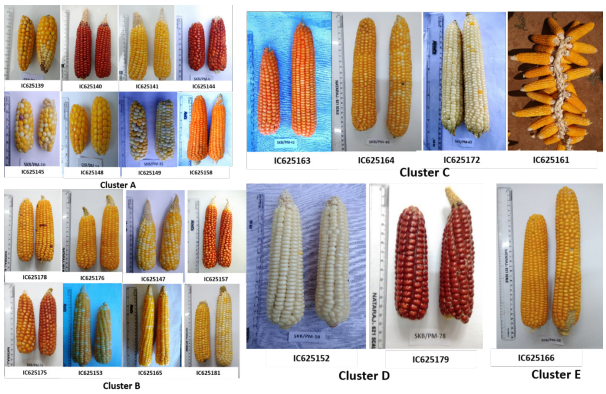

Figure 1: Phenotypic diversity in ears of maize germplasm accessions collected from Chhattisgarh, India

Table 1: The passport details of maize accessions collected from Chhattisgarh

Sr. No.	Collector No.	IC No.	Landrace name	Kernel color
1	SKB/PM-1	IC625139	Makai	Yellow
2	SKB/PM-5	IC625140	Makai	Purple
3	SKB/PM-6	IC625141	Makai	Yellow and few white kernels
4	SKB/PM-7	IC625142	Dehati makka	Yellow and few white kernels
5	SKB/PM-8	IC625143	Jowari makka	Yellow with many white kernels
6	SKB/PM-9	IC625144	Lal makka	Purple
7	SKB/PM-10	IC625145	Makai	Pale, white and few purple kernels
8	SKB/PM-11	IC625146	Makai	Yellow
9	SKB/PM-12	IC625147	Makai	Yellow with many white kernels
10	SKB/PM-14	IC625148	Makai	Yellow
11	SKB/PM-15	IC625149	Dehati Makai	Pale and white
12	SKB/PM-16	IC625150	Dehati Makka	Pale
13	SKB/PM-17	IC625151	Dehati Makka	Yellow and white kernels
14	SKB/PM-18	IC625152	Desi Makka	White
15	SKB/PM-19	IC625153	Pili Makka	Yellow with many white kernels
16	SKB/PM-21	IC625154	Dehati Makai	White with many pale kernels
17	SKB/PM-22	IC625155	Makai	Yellow
18	SKB/PM-24	IC625156	Makai	Pale
19	SKB/PM-28	IC625157	Lal makai	Orange with a purple tinge
20	SKB/PM-31	IC625158	Makka	Orange
21	SKB/PM-35	IC625159	Bhutta Makka	Yellow
22	SKB/PM-36	IC625160	Makka	Yellow and white
23	SKB/PM-39	IC625161	Makka	Yellow
24	SKB/PM-40	IC625162	Makka	Yellow with white tinge
25	SKB/PM-42	IC625163	Makka	Orange
26	SKB/PM-46	IC625164	Makka	Yellow
27	SKB/PM-47	IC625165	Makka	Yellow with few white kernels
28	SKB/PM-48	IC625166	Makai	Yellow
29	SKB/PM-50	IC625167	Makai	Orange
30	SKB/PM-54	IC625168	Makai	Yellow
31	SKB/PM-56	IC625169	Makai	Orange
32	SKB/PM-58	IC625170	Makai	yellow
33	SKB/PM-61	IC625171	Makai	Separate orange and yellow ears
34	SKB/PM-63	IC625172	Safed Makka	White with few yellow kernels
35	SKB/PM-66	IC625173	Makka	Orange with few purple kernels
36	SKB/PM-71	IC625174	Pila makka	Yellow with few white kernels
37	SKB/PM-73	IC625175	Makka	Light purple
38	SKB/PM-75	IC625176	Makai	Yellow
39	SKB/PM-76	IC625177	Bhusri makka (Pila)	Purple
40	SKB/PM-77	IC625178	Bhusri makka (Safed)	Yellow with few purple kernels
41	SKB/PM-78	IC625179	Makai	Purple
42	SKB/PM-79	IC625180	Makai	Variegated
43	SKB/PM-83	IC625181	Makai	Separate yellow and white with many yellow kernels

observed to be either mostly long and thin with a broad base and having yellow kernels or short but compact with yellow or orange-colored kernels. The numbers of kernel rows per ear were found to be of even numbers for all the collected accessions and ranged from 8–18 rows per ear.

The descriptive statistics (mean, range, standard error of mean, standard deviation and coefficient of variation) were computed for all the quantitative variables based on the data recorded on six quantitative traits for 43 accessions collected from Chhattisgarh and it is presented in Table 2.

Based on the quantitative range of the studied trait *viz.* ear length (7.33–26.67 cm), ear girth (8–16.33 cm), number of kernel rows per ear (8.00–18.00), number of kernels per ear row (12.67–45.33), ear weight (25.45–204.88 g) and 100 kernel weight (9.74–38.66 g), with the range of each trait given in the parenthesis, it was concluded that significant intra-trait variability was present in the collected accessions. The coefficient of variation, which is the ratio of standard deviation to the mean and represents the extent of variability in a trait relative to the population mean was maximum for ear weight (43%) followed by 100 kernel weight (26.34%), ear length (26.26), number of kernels per ear row (25.92%), number of kernel rows per ear (17.71%) and ear girth (17.10%). Based on the computed CV, the traits can be classified into highly variable (ear weight), moderately variable (100 kernel weight, ear length, number of kernels per ear row) and relatively less variable or stable (number of kernel rows per ear and ear girth). The statistical inferences drawn on *in-situ* germplasm characterization were utilized to identify trait-specific superior accessions even before the actual comprehensive evaluation exercise of the collected accessions (Table 3). The maximum ear length (26.67 cm), ear girth (16.33 cm), number of kernel rows per ear (18), number of kernels per ear row (45), ear weight (204.88 g) and 100 kernel weight (38.66 g) were recorded for the accessions IC625166, IC625161, IC625169 and IC625161, IC625173, IC625167 and IC625152 respectively. However, maize being a highly cross-pollinated crop the multi-location and multi-year characterization of superior accessions is required for validation of the findings of the present study.

Table 2: Descriptive statistics for six quantitative traits in 43 maize accessions collected from Chhattisgarh, India

Statistics	*Ear length (cm)*	*Ear diameter (cm)*	*Rows/ ear*	*Kernels/ row*	*Ear weight (g)*	*100 Kernel weight (g)*
N	43.00	43.00	43.00	43.00	43.00	43.00
Min.	7.33	8.00	8.00	12.67	25.45	9.74
Max.	26.67	16.33	18.00	45.33	204.88	38.66
Mean	15.05	11.64	13.40	29.22	112.88	20.45
SE	0.60	0.30	0.36	1.16	7.40	0.82
Variance	15.61	3.97	5.63	57.40	2356.23	29.01
SD	3.95	1.99	2.37	7.58	48.54	5.39
CV%	26.26	17.10	17.71	25.92	43.00	26.34

Table 3: Trait-wise superior accessions were identified among 43 maize accessions collected from Chhattisgarh, India.

S. No.	*Character*	*Superior accessions*
1	Ear length (cm)	IC625166 (26.67), IC625171(20.67), IC625173 (20.67)
2	Ear girth (cm)	IC625161 (16.33), IC625164 (16.00), IC625169 (15.33)
3	Rows/ear	IC625169 (18), IC625161 (18), IC625162 (16)
4	Kernels/row	IC625173 (45), IC625163, (43), IC625166 (43)
5	Ear weight (g)	IC625167 (204.88), IC625166 (197.19), IC625163 (196.44)
6	100 kernels weight (g)	IC625152 (38.66), IC625166 (30.82), IC625179 (31.44)

Principle Component Analysis

Principle component analysis (PCA) is routinely used in germplasm characterization trials for quantification of the genetic variability based on the phenotypic traits, and thus it helps to identify the breeding value of a genotype. PCA analysis was carried out to classify the collected maize genotypes on the basis of the most discriminatory traits.

The Eigen values, percent variance and cumulative variance were estimated for six quantitative traits using PAST software. The first three components explained 99.61% of the total variation in the set of maize landraces collected from Chhattisgarh (Table 4).

The first component explained 97.33% of the total variation followed by PC II, PC III, PC IV, PC V and PC VI which explained 1.67, 0.61, 0.19, 0.13 and 0.06% of the total variation, respectively. The major contributing trait for PC I was ear weight with 0.99 loading factor and the remaining of the traits contributed positively but with very small loadings. Ear weight loaded negatively for remaining of the five principal

Table 4: The Eigenvalues, the proportion of the total variance (%) represented by first six principle components, cumulative variance (%) and component loading of six quantitative traits in 43 maize landraces collected from Chhattisgarh, India.

Trait	PC I	PC II	PC III	PC IV	PC V	PC VI
Ear length	0.06	0.33	0.16	-0.01	0.84	0.40
Ear girth	0.02	0.08	-0.04	0.58	0.33	-0.74
Rows/ear	0.02	0.01	-0.35	0.75	-0.18	0.53
Kernels/row	0.10	0.90	0.19	0.03	-0.38	-0.05
Ear weight	0.99	-0.09	-0.09	-0.05	-0.01	-0.02
100 kernel weight	0.08	-0.26	0.90	0.31	-0.13	0.11
Eigen value	2401.99	41.26	15.04	4.78	3.18	1.59
Variance (%)	97.33	1.67	0.61	0.19	0.13	0.06
Cumulative variance (%)	97.33	99.00	99.61	99.81	99.94	100.00

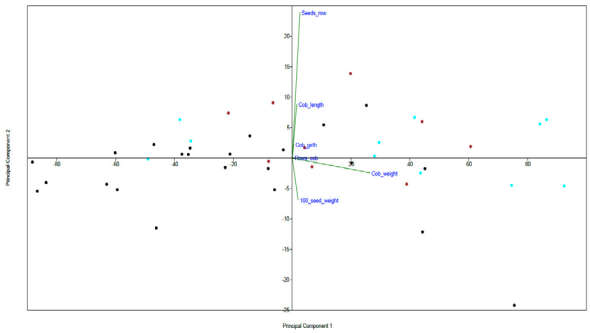

Figure 2: Scatter plot derived from the PCA analysis of 6 quantitative traits of maize

components. The trait number of kernels per ear row (0.90) contributed maximally towards the variability present in PC II and loaded negatively for PC V and PC VI. The 100-kernel weight (0.90) was the major loading trait for PC III and it loaded negatively for PC II and PC V. The number of kernel rows per ear (0.75), loaded maximum for PC IV and negative for PC III and V. The ear length (0.84) loaded positively and maximum for PC V and negatively for PC IV. For PC VI number of kernel rows per ear loaded maximum (0.53) while ear girth loaded negatively for PC III and PC VI. During the generation advancements, the positively loading traits are expected to have high propensity of being inherited together and vice versa. The PCA plotted using first two principal components (Figure 2) showed that the germplasm collected from three different districts are diverse and even within a single district, the genotypes were distributed away from each other. Among the collected accessions, ear weight was the most significant contributing trait to the total variability. The character association was performed by estimating the correlation coefficients for all combinations of six quantitative traits (Table 5). A highly significant positive correlation was found between most of the traits except a negative non-significant correlation between number of kernel rows/ear and 100 kernel weight, indicative of a strong inherent association between the studied traits.

Table 5: Correlation among different traits in maize accessions collected from Chhattisgarh, India

Trait	*Ear length*	*Ear girth*	*Kernel rows/ear*	*Kernels/ ear-row*	*Ear weight*	*100 kernel weight*
Ear length	-					
Ear girth	0.52**	-				
Kernel rows/ ear	0.13*	0.46**	-			
Kernels/ear-row	0.85**	0.47**	0.17**	-		
Ear weight	0.71**	0.45**	0.32**	0.62**	-	
100 kernel weight	0.42**	0.24**	-0.06	0.27**	0.68**	-

Genetic Diversity Analysis (Cluster analysis)

The cluster analysis based on the Euclidean distances estimated on the quantitative traits was conducted in the present study. The Euclidean distance is only appropriate for data measured on the same scale which holds good for the present study. A dendrogram was constructed based on the estimated Euclidian distances among all the collected maize germplasm accessions (Figure 3). The cluster analysis grouped the 43 maize accessions into five distinctive groups *viz*. A, B, C, D, and E with each cluster having 9, 18, 13, 2 and 1 accessions. Cluster A had eight accessions originating from the district Jaspur while only one accession originating from Korba while cluster B had 11 accessions from Jaspur, four accessions from Koriya and three accessions from the Korba districts, making it geographically most divergent cluster as far as the collection sites are concerned. The accessions clustered together in cluster C originated mostly from Korba (6 accessions) and Koriya (5 accessions) districts with only two accessions originating from the Jaspur district. The cluster D had only two accessions, both from the Jaspur district, while cluster E comprised only one accession originating from the Korba district. The clusters B and C had genotypes originating from all three districts. However, there was observed a linearity of the spread among accessions clustering together i.e., the accessions collected consecutively on a geographic trail clustered together irrespective of the administrative district boundaries and geographical attributes of the collection sites, which can be understood in terms of germplasm diffusion along narrow geographic tracks. The number and percent of genotypes in each cluster and their associated phenotypic characteristics pertaining to the six quantitative traits recorded in the present study is presented in Table 6. Genotypes located in different clusters should be considered for genetic improvement of maize, particularly as components of heterotic pools in the hybrid breeding programs. The cluster means and standard deviation for six quantitative traits in maize germplasm accessions collected from Chhattisgarh, India is given in Table 7. The genotypes

Table 6: Clustering together and phenotypic characteristics of 43 maize accessions collected from Chhattisgarh

Cluster	*No. of genotypes*	*Percentage*	*Phenotypic characteristics*
A	9	20.93	Short ears, low ear girth, medium number of kernel rows per ear, low number of kernels per row, lowest ear weight, lowest seed weight, collected from medium elevation.
B	18	41.86	Medium ear length, lowest ear girth, lowest number of kernel rows per ear, medium number of kernels per row, medium ear weight, medium 100 kernel weight, collected from medium elevation.
C	13	30.23	High ear length, medium to high ear girth, highest number of kernel rows per ear, medium to high number of kernels per ear row, medium ear weight, low to medium 100 kernel weight, collected from medium elevation.
D	2	4.65	Low ear length, low ear girth, low kernel rows per ear, low number of kernels per ear row, high ear weight, highest 100 kernel weight, collected from high elevation.
E	1	2.33	Highest ear length, highest ear girth, high number of kernel rows per ear, highest number of kernels per ear row, highest ear weight, high hundred kernel weight, collected from low elevation.

clustered together in cluster C had maximum average ear length (18.26 ± 2.09), ear girth (13.41 ± 1.45), kernel rows per ear (15.08 ± 2.10) and number of kernels per row (36.59 ± 4.23) while cluster D had highest average ear weight (173.28 ± 22.47) and highest average 100 kernel weight (35.05 ± 5.11) with the trait mean ± standard deviation given in the parenthesis. The cluster E had only one genotype, so average and standard deviation could not be computed. However, singly it ranked first in all the traits except 100 kernel weight. The clustering together of genotypes resulted due to the distinct phenotypic traits utilized for cluster analysis.

Geo-spatial Analysis of Genetic Diversity

The grid maps (20 minute raster cell) were generated for diversity analysis of the six quantitative traits *viz*. ear girth, ear weight, ear length, number of kernel rows per ear, number of kernels per ear row and 100 kernel weight in the collected maize germplasm accessions (Figure 4). The grid colors are indicative of extent of trait-specific diversity in a particular geographic location in the explored areas. The diversity pattern of maize germplasm accessions revealed by the grid maps confirmed the existence of considerable genetic diversity for all six quantitative traits in the surveyed area.

The maize germplasm accession IC624139 with the smallest ears (7.33 cm) and eventually minimum ear weight (25.45) was collected from Jaspur district and that with the longest ear IC625166 (26.67 cm) was collected from Korba district of the Chhattisgarh state. The ear girth was found to be minimum (8 cm) in IC625180 collected from Jaspur and a maximum (16.33 cm) in IC625161 collected from Koriya. The number of kernel rows per ear were a minimum (8) in the accession number IC625142 collected from Jaspur and a maximum (18) in two accessions (IC625161 and IC625168) both collected from Korba district. The number of kernels per ear row was minimum (12.67) in IC625149 collected from Jaspur and a maximum (45.33) in IC625173 collected from Koriya. The ear weight was recorded to be maximum (204.88 g) in accession number IC625167 collected from Korba. The accessions with minimum (9.74) and maximum (38.66 g) 100 kernel weights, i.e., IC625144 and IC625152, were collected from Jaspur district.

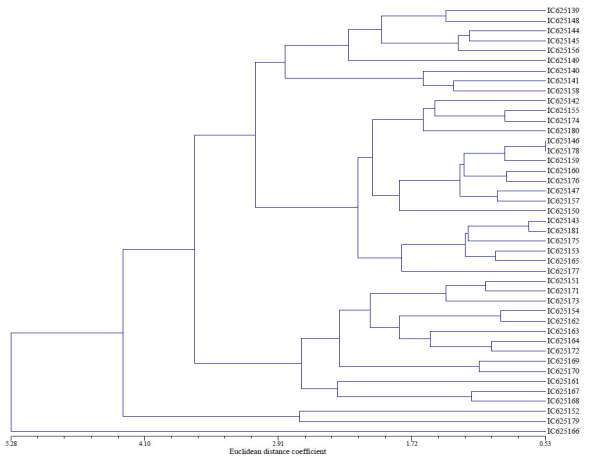

Figure 3: Dendrogram generated based on quantitative traits of 43 maize genotypes based on Euclidean distances

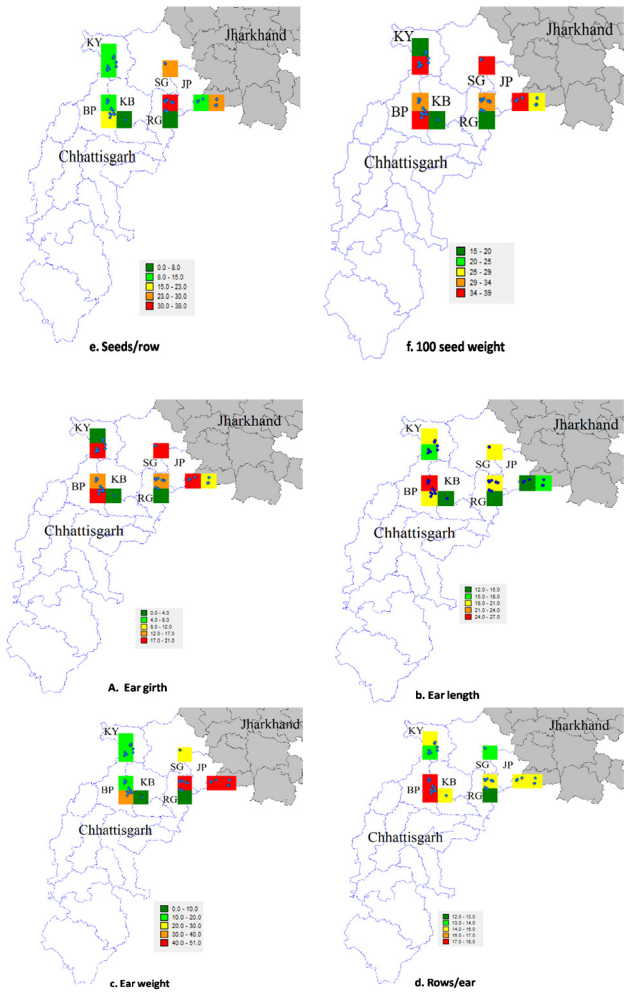

Figure 4: Trait-wise geo-referenced source sites for maize germplasm from Chhattisgarh, India

Table 7: Cluster means and standard deviation for six quantitative traits in maize germplasm accessions collected from Chhattisgarh, India

	Ear length (cm)	*Ear girth (cm)*	*Rows/ear*	*Kernels/row*	*Ear weight*	*100 kernel weight*	*Average elevation*
Cluster A	10.52 ± 2.64	11.33 ± 1.76	14.22 ± 2.11	19.56 ± 4.30	57.60 ± 28.08	15.12 ± 3.05	556.88 ± 141.47
Cluster B	14.61 ± 2.11	10.28 ± 1.10	11.89 ± 1.75	28.98 ± 2.68	101.93 ± 30.29	20.51 ± 3.29	576.83 ± 221.83
Cluster C	18.26 ± 2.09	13.41 ± 1.45	15.08 ± 2.10	36.59 ± 4.23	150.54 ± 34.21	21.02 ± 3.42	540.85 ± 161.16
Cluster D	12.67 ± 1.89	11.67 ± 1.41	12.00 ± 2.83	20.17 ± 4.95	173.28 ± 22.47	35.05 ± 5.11	1086.00 ± 4.24
Cluster E	26.67	16.00	14.00	43.00	197.19	30.82	488

The grid maps generated by DIVA-GIS successfully explained the existence and distribution of trait specific diversity in the maize germplasm in the explored regions of the Chhattisgarh state. Overall, the district of Jaspur has germplasm diversity with small ear size, smaller ear girth, a smaller number of kernel rows per ear and a smaller number of kernels per ear. However, the diversity for both small seed size and bold seeds was observed and collected from this district. The maize germplasm diversity for long ear length, a greater number of kernel rows per ear and high ear weight was found to be present in the Korba district while the district of Koriya exhibited diversity for high ear girth and number of kernels per ear row.

It could be concluded that the diversity of maize germplasm in the explored area is spatially dispersed and different regions have evolved different traits may be due to adaptation to differential eco-geographies or farmer's preferences. The similar eco-geographies may be targeted elsewhere in the country for collection, conservation and utilization of specific traits in the maize improvement programmes, particularly for introgression of various adaptive traits in the hybrid development for targeted areas. Most of the germplasm accessions were acquired from the difficult terrain regions where the cropping intensity is very low and they are comprised of subsistence small scale agriculture which possesses the non-improved local indigenous cultivars. Therefore, the primary objective of the study to target the interior diversity pockets with primitive agriculture for collection of local unimproved germplasm/landraces/farmer's varieties and their subsequent conservation, thus, stood fulfilled.

Discussion

The eco-geographic attributes and socioeconomic variables are the main determining factors influencing the distribution pattern of crop genetic diversity in a particular area through the process of local adaptation (Vilaro *et al.,* 2020; Vidal *et al.,* 2020). The agricultural significance of the locally adapted crop landraces can only be realized through their collection and their subsequent use as a source of genes or their refinement through exercising selection on this variability (Casanas *et al*., 2017). In this context, the sampling technique employed assumes great significance because effective sampling is the one that takes into account all these above-mentioned parameters. Recently, Vidal *et al*. (2020) have described a sampling methodology named the maximization method that successfully captured the maximum variability evaluated in the diversity census from the smallest sample size in maize.

The present study, which was aimed at the exploration and collection of maize landraces diversity from the Eastern Indian state of Chhattisgarh, resulted into a collection of 43 highly diverse accessions as reflected in their unique morphological attributes. Kumar *et al*. (2015a) have attributed the existence of high morphological variation in the maize landraces collected from the North-Western Himalayan region to the open-pollinated nature of local accessions, their adaption to local conditions and the continuous use of seeds by the farmers.

Most of the collections pertaining to the present study fell into late maturing flint type maize genotypes, indicating that this trait has had an adaptive advantage in the localized germplasm acclimatization. The evolution and perpetuation of late-maturing genotypes in North-Eastern India has been previously described by Singh and Singh (1967), and further validated by Singh (1977). The late maturing genotypes have been endowed with inherent drought tolerance ability. As such, it is considered to be the reason behind the evolution and perpetuation of late-maturing genotypes. Moreover, the late maturing accessions are at an advantage in having higher values of grain weight, tryptophan content, node length, tassel length and ear length, translating into higher yield as well as quality (Kumar *et al.,* 2015a). It was found in the present study that the altitudinal elevation of the geographic sites of cultivation systems have a definite bearing on the cultivation/non-cultivation of specific maize landraces in a particular area. This finding is in agreement with those of Singh (1977) who concluded that altitude, being a complex geographic barrier, is the main factor influencing the distribution of Indian races of maize. Mercer *et al.* (2008) found that the adjusted fitness of the highland landraces of Mexico was greater than that of the midland landraces under highland conditions. It was also reported that the landraces being cultivated under lower elevations are more prone to replacement by improved varieties/hybrids than the highland landraces. However, climate change makes the highland landraces more prone to temperature rise. Therefore, the prioritization of the collected landraces in the present study for utilization in the breeding programmes and further micro-level diversity collection should be undertaken while taking into consideration the elevation of their collecting sites.

The results of the principal component analysis are in agreement with those of Sharma *et al.* (2010) who found that 100-kernel weight, ear length, ear girth, number of kernels per ear, grain yield and days to flowering were the major differentiating traits in PCA conducted to differentiate the 'Sikkim Primitives' from the rest of the accessions in maize genotypes from North-Eastern India. Kumar *et al.* (2015a) have also reported the important role of kernel rows per ear as a major differentiating trait, among others. Similarly, Kumari *et al.* (2017) in their study comprising of characterization of maize landraces originating from North-Eastern India have also reported the high discriminatory power of the traits, days to tasseling, days to silking, ear length, ear weight, days to maturity and grain yield, kernels per ear row, kernel rows per ear and kernel weight which are in agreement with the present study. The results obtained by Dar *et al.* (2018), i.e. ear

weight being the most important trait contributing to the total genetic variability present in the germplasm set and as the most loading trait in the first principle component, also substantiate the present study's findings.

Character Association

The results of the inter-trait correlation-based character association are in agreement with Kumar *et al.,* (2015b), who reported that the traits plant height, ear height, ear length, ear girth, number of kernel rows per ear, number of kernels per row and 100 kernels weight registered positive and significant correlation with each other. Similar findings for significant positive correlation among different yield-attributing traits have also been reported by Shukla (2017). The correlation analysis conducted by Kumar *et al.* (2015a), revealed that the phenological parameters had a positive influence on maize kernel weight, tryptophan content, node length, tassel length and ear length. The study provided insight for the reason for the perpetual of the late maturity genotypes as the late maturing accessions will have higher values of these traits, meaning enhanced yield and quality.

Cluster Analysis

The cluster analysis findings in the present study do not agree with those of Kumari *et al.* (2017), who reported the clustering of maize accessions from North-Eastern India according to their geographical sites of origin. Similarly, Kumar *et al.* (2015a) also obtained clustering together of accessions depending on the site of origin and the linearity of the spread of diversity as observed in the present study, was absent. The finding in the present study indicates the natural gene flow as well as diffusion of germplasm in the shared terrain and linear eco-geography. Therefore, it may be implied that the genotypes clustered together should ideally have more genetic relatedness than those clustered in different clusters. In the cluster analysis studies conducted by Prasanna *et al.* (2009), the 'Sikkim Primitives' clustered separately from the accessions from elsewhere in India and it was attributed to the alleles associated with the unique features of the 'Sikkim Primitives'. Similar results were reported by Sharma *et al.* (2010), where accessions from Sikkim formed a discrete cluster, indicating their uniqueness among maize landraces in India based on the similarity of phenotypic attributes among accessions that clustered together. Dar *et al.* (2018) have also reported similar cluster analyses based on morphological characterization of maize accessions collected from the North-Western Himalayan region.

The present study is the maiden attempt on cluster analysis on the germplasm originating from elsewhere in India's North-Eastern or North-Western Himalayan regions. Dar *et al.* (2018) evaluated a diverse set of maize inbred lines sourced from all over India, using DIVA-GIS and through generation of grid maps, successfully locating the sites from which trait-specific germplasm for eleven quantitative traits can be sourced. Kumari *et al.* (2017) generated grid maps for the diversity index of 100 seed weight in maize accessions collected from western, central and southern parts of Nagaland state of India using DIVA-GIS software and identified geographic locations exhibiting diversity for this important yield-attributing trait. Various workers already use the DIVA-GIS tool for geo-referencing the diversity of different important crops. Jatropha (Sunil *et al.,* 2009), linseed (Sivaraj *et al.,* 2009, 2012), black gram (Babu *et al.,* 2010), onion (Kamala *et al.,* 2011) and common bean (Sultan *et al.,* 2014), have not been utilized to its full potential for plant genetic resources management in India to fully understand the distribution of diversity of major crops on the geographical scale (Dar *et al.,* 2018). As such, the present study revealed many new information about the adaptation and evolution of maize germplasm from a previously little-explored area of India.

Conclusion

The present study resulted in the collection and conservation of genetically diverse maize landraces besides the identification of several trait-specific superior accessions and their geo-referenced collection sites for further prioritization and acquisition of the landraces that might possess unique traits. These accessions can be utilized as potential sources of novel allelic combinations using novel cross-disciplinary research tools such as genoplasmics, genome wide association studies, genomic selection, and allele mining.

The collected accessions represent the sub-tropical maize germplasm and can be utilized for sub-tropical x temperate crosses for introgression of adaptiveness in the exotic temperate cultivars, which perform poorly in the sub-tropical Indian conditions. The study should also be helpful to future maize germplasm explorers and collectors for understanding the geographic patterns of genetic diversity within germplasm accessions for effective and efficient germplasm acquisition, conservation and utilization.

Acknowledgements

The authors sincerely thank the farmer conservators of the Chhattisgarh state for their custodianship of the precious diversity of maize, the Krishi Vigyan Kendra of Jaspur, Koriya and Korba for their help in locating and collecting the diversity and Dr. Kuldeep Singh, Director, ICAR-NBPGR, Pusa Campus, New Delhi for the research facilities.

References

Azeez MA, AO Adubi and FA Durodola (2018) Landraces and Crop Genetic Improvement. Rediscovery of Landraces as a Resource for the Future. *doi: 10.5772/intechopen.75944*

Babu A, V Kamala, N Sivaraj, N Sunil, NSR Pandravada, M Vanaja and KS Varaprasad (2010) DIVA-GIS approaches for diversity assessment of pod characteristics in black gram (*Vigna mungo* L. Hepper). *Curr Sci.* **98:** 616-619.

BDA (2002) *http://nbaindia.org/uploaded/Biodiversityindia/Legal/31.%20Biological%20Diversity%20%20Act,%202002.pdf*

Brown AHD and JD Briggs (1991) Sampling strategies for genetic variation in ex situ collections of endangered plant species. In: Falk DA and Holsinger KE (eds). Genetics and Conservation of Rare Plants. Oxford University Press, New York. pp 99–119.

Brown AHD and DR Marshall (1995) A basic sampling strategy: theory and practice. In Collecting Plant Genetic Diversity. Technical Guidelines. Editors Guarino, L., Rao, R.V. e Reid, R. Cab International, Walligford. pp.75-92.

Casañas F, J Simó, J Casals and J Prohens (2017) Toward an evolved concept of landrace. *Front. Plant Sci.* 08. *doi:10.3389/fpls.2017.00145*

Dar TH, R Shakeel and S Verma (2018) Comparative Germplasm Characterization of Maize (*Zea mays* L.) in Rajouri Region of Pir Panjal Himalaya J & K (India), based on Morphological and ISSR Markers. *J. Crop Sci. Biotechnol* **21(1):** 43-55.

Delucchi C, GH Eyhérabide, RD Lorea, DA Presello, ME Otegui and CG López (2012) Classification of argentine maize landraces in heterotic groups. *Maydica* **57:** 26-33.

Dwivedi SL, S Ceccarelli, MW Blair, HD Upadhyaya, AK Are, and R Ortiz (2016) Landrace germplasm for improving yield and abiotic stress adaptation. *Trends Plant Sci.* **21(1):** 31–42.

Hammer Q, David ATH and Paul DR (2001) Past:Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol Electron* **4(1):** 9.

Hijmans RJ, L Guarino and P Mathur (2012) DIVA-GIS Version 7.5 Manual. University of California Davis, California, USA (Manual available at *http://www.diva-gis.org/).http://pgrportal.nbpgr.ernet.in/(S(du0aqt55fzhd11553m104v45))/default.aspx*

Kamala V, AJ Gupta, N Sivaraj, SR Pandravada, N Sunil, KS Varaprasad, KE Lawande (2011) Diversity analysis of onion germplasm collections from north Telangana region of Andhra Pradesh. *Indian J. Plant Genet. Resour.* **24(2):** 163-171.

Kumar A, J Kumari, JC Rana, DP Chaudhary, R Kumar, H Singh, TP Singh and M Dutta (2015a) Diversity among maize landraces in North West Himalayan region of India assessed by agro-morphological and quality traits. *Indian J. Genet. Plant Breed.* **75(2):** 188-195.

Kumar V, SK Singh, PK Bhati, A Sharma, SK Sharma and V Mahajan (2015b) Correlation, Path and Genetic Diversity Analysis in Maize (*Zea mays* L.). *J. Ecol. Environ.* **33(2A):** 971-975.

Kumari J, A Kumar, TP Singh, KC Bhatt, AK Mishra, DP Semwal, RK Sharma and JC Rana (2017) Collection, evaluation and phenotypic diversity assessment of maize (*Zea mays*) germplasm from North Eastern Himalayan region. *Indian J. Agri. Sci.* **87(6):** 727–33.

Mir RA, A Sharma and R Mahajan (2020) Crop Landraces: Present threats and opportunities for conservation. In: Salgotra R., Zargar S. (eds) Rediscovery of Genetic and Genomic Resources for Future Food Security. *Springer*, Singapore. *https://doi.org/10.1007/978-981-15-0156-2_13*

Mercer K, A Matínez-Vásquez and HR Perales (2008) Asymmetrical local adaptation of maize landraces along an altitudinal gradient. *Evol. Appl.* **1:** 489-500.

Pandey A, DP Semwal, SP Ahlawat and SS Sharma (2015) Maize (*Zea mays*): Collection Status, Diversity Mapping and Gap Analysis. National Bureau of Plant Genetic Resources, New Delhi, India, pp. 34.

PPVFR&A (2001). *https://www.indiacode.nic.in/bitstream/123456789/1909/1/200153.pdf*

Prasanna BM (2012) Diversity in global maize germplasm: Characterization and utilization. *J Biosci* **37:** 843-855.

Prasanna BM (2010) Phenotypic and molecular diversity of maize landraces: characterization and utilization. *Indian J. Genet. Plant Breed.* **70:** 315-327.

Prasanna BM, L Sharma, SK Wasala, A Singode, R Kumar, SK Guleria, JC Sekhar, R Karuppaiyan, K Srinivasan and HS Gupta (2009) Maize landraces in India-Phenotypic and molecular characterization: *ICAR Newsletter* pp. 1-3.

Rohlf FJ (2005) NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.2. Exeter Software, Setauket, NY.

Sharma L, BM Prasanna and B Ramesh (2010) Analysis of phenotypic and microsatellite-based diversity of maize landraces in India, especially from the North East Himalayan region. *Genetica* **138:** 619-631.

Shukla N (2017) Genetic variation and association study for grain yield in germplasm accessions of maize (*zea mays* L.). *Indian J. Agric. Res.* **51:** 120-127.

Singh B (1977) Races of Maize in India. Indian Council of Agricultural Research (ICAR), New Delhi.

Singh BP and Srivastava U (2004) Plant Genetic Resources in Indian perspective: Theory and practices. Indian Council of Agricultural Research, New Delhi. ISBN 81-7164-017-6.

Singh S and J Singh (1967) Composite populations developed from Indian maize varieties, paper submitted to the annual maize improvement conference held at Indian Agricultural Research Institute, New Delhi.

Sivaraj N, N Sunil, SR Pandravada, V Kamala, K Vinod, A Babu, BVSK Rao, RBN Prasad and KS Varaprasad (2012) Variability in linseed (*Linum usitatissimum*) germplasm collections from peninsular India with special reference to seed traits and fatty acid composition. *Indian J. Agric. Sci.* **82 (2):** 102-105.

Sivaraj N, N Sunil, SR Pandravada, V Kamala, K Vinod, BSK Rao, RBN Prasad and KS Varaprasad (2009) DIVA-GIS approaches for diversity assessment of fatty acid composition in linseed (*Linum usitatissimum* L.) germplasm collections from peninsular India. *J. Oilseeds Res.* **26:** 13-15.

Steel RGD and JH Torrie (1980) Principles and Procedures of Statistics. A biometrical approach. 2nd edition. McGraw-Hill, New York, USA. pp. 20-90

Sultan SM, SA Dar, SA Dand and S Natarajan (2014) Diversity of common bean in Jammu and Kashmir, India: A DIVA-geographic information system and cluster analysis. *J. Appl. Nat. Sci.* **6:** 226-33.

Sunil N, N Sivaraj, K Anitha, A Babu, KE Vinod, M Sudhir, Vanaja and KS Varaprasad (2009) Analysis of diversity and distribution of Jatropha curcas L. germplasm using Geographic Information System (DIVA-GIS). Genet. Resour. *Crop Evol.* **56:** 115-119.

Tokatlidis I and D Vlachostergios (2016) Sustainable Stewardship of the Landrace Diversity. Diversity **8(4):** 29. *doi:10.3390/d8040029*

Vidal R, NC de A Silva and JB Ogliari (2020) Old tools as new support for on farm conservation of different types of maize. *Sci Agric.* **77(1):** *doi:10.1590/1678-992x-2018-0091*

Vilaró M, R Vidal and T Abadie (2020) Diversity of Maize landraces in germplasm collections from South America. *Agrociencia Uruguay* **23(1):** 108. *https://doi.org/10.31285/AGRO.24.108*

Yadav OP, F Hossain, CG Karjagi, B Kumar, PH Zaidi, SL Jat, JS Chawla, J Kaul, KS Hooda, P Kumar, P Yadava and BS Dhillon (2015) Genetic improvement of maize in India-retrospect and prospects. *Agric. Res:* **4:** 325-38.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2023.00036.2.06

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Phenotypic Characterization and Grouping of Rose (*Rosa* × *Hybrida* L.) Genotypes Using DUS Descriptors

Aparna Veluru[1*], Kangila V. Bhat[2], Janakiram Tolety[3], Venkata P. Kuchimanchi[4], Venkata S. R. Dantuluri[4], Sapna Panwar[5], Namita[5] and Kanwar P. Singh[5]

Abstract

Characterization and evaluation of native germplasm is essential for improvement, protection and conservation. The present investigation was carried out to study the phenotypic diversity of 148 rose (*Rosa* × *hybrida* L.) genotypes at ICAR-Indian Agricultural Research Institute, New Delhi during two consecutive years (2018-19 and 2019-20). Characteristics (48) were used to identify and group the genotypes. Phenotypic data of genotypes was collected during peak flowering season by following DUS (distinctiveness, uniformity and stability) test guidelines developed by PPV & FRA, India. The DUS characteristic profile was made of the all 148 genotypes using 48 phenological traits. Hierarchical cluster analysis was conducted for the grouping of studied genotypes. A total of eight different clusters were identified in the dendrogram with high characteristic uniformity. Cluster-I is identified with a maximum number of genotypes (109), followed by cluster-III (11), cluster- II and VIII (7 each), cluster-IV &VII (5 each), cluster-V (3) and cluster-VI (1). This information about an existing huge varietal wealth of rose will be useful for researchers, farmers, nursery men to pursue protection for their new material under PPV and FRA, New Delhi.

Keywords: Rose, Germplasm, Phenotypic diversity, Characterization, DUS.

[1]Division of Crop Improvement, ICAR-CPCRI, Kasaragod, Kerala, India

[2]ICAR-National Bureau of Plant Genetic Resources, New Delhi, Delhi, India

[3]Vice Chancellor, Dr. YSR Horticultural University, Andhra Pradesh, India

[4]ICAR-Directorate of Floricultural Research, Pune, Maharashtra, India

[5]Division of Floriculture and Landscaping, ICAR-IARI, New Delhi, Delhi, India

***Author for correspondence:**

aparna.cpcri@gmail.com

Received: 01/06/2022 **Revised:** 18/12/2022

Accepted: 19/12/2022

How to cite this article: Veluru, A., Bhat, K.V., Tolety, J., Kuchimanchi, V.P., Dantuluri, V.S.R., Panwar, S., Namita5, Singh, K.P. (2023). Phenotypic Characterization and Grouping of Rose (*Rosa* × *Hybrida* L.) Genotypes Using DUS Descriptors. *Indian J. Plant Genetic Resources*. 36(2), 243-249. **DOI:** 10.5958/0976-1926.2023.00036.2.06

Introduction

Morphological characterization is the traditional approach used for the classification of existing germplasm and assessing variability. Though phenotypic traits are considered as primitive markers, they are powerful indeed to determine the taxonomic status of any life form and scientific grouping of the plant kingdom still relies on morphological traits. Genetic variability in roses was assessed by using morphological markers (Panwar *et al*., 2010; Gaurav *et al*., 2018; Aparna *et al*., 2019; Aparna *et al*., 2020). Different national and international IPR (Intellectual Property Rights) authorities regulating plant varieties and breeder's rights (UPOV, PPV&FRA, etc.) considered the phenotypic characteristics (DUS characteristics) as the basis for variety or genotype registration in order to provide protection to the material. Protection of Plant Varieties and Farmers Rights Authority (PPV&FRA), India developed DUS test guidelines considering 61 and 16 phenotypic characteristics for *Rosa* spp. (including varieties, hybrids, and wild genotypes) and *R. damascena,* respectively. In addition to identifying organisms, morphological characterization helps in studying genetics, taxonomy, evolutionary developments, measuring diversity and determining the material's economic usage, etc. Therefore, the present study was taken up with the objective of probing existing diversity within in Indian rose germplasm. In the current study, 148 rose genotypes, including wild species, were taken and were assessed for their variability using 59 DUS descriptors developed by PPV& FRA, India.

Materials and Methods

The study was carried out at division of Floriculture and Landscaping, ICAR- IARI, New Delhi, during two successive flowering seasons (February-March) of 2017-18 and 2018-19. The experimental material consisted of 148 rose genotypes (Table 1). The selected genotypes (10 plants/genotype) were maintained in the field at a spacing of 60 × 60 cm and a uniform package of practices were followed in genotypes to nullify the statistical errors. The data on 59 different morphological characters were collected from the selected genotypes (10 plants/genotype) during two consecutive years (2018 and 2019) based on PPV & FRA drafted DUS guidelines of roses (PPV & FRA, 2009) (Table 2). Qualitative and pseudo-qualitative characteristic parameters were recorded by visual assessment and quantitative parameters were measured physically under daylight conditions on ten plants per replication by excluding border rows. The observations related to color was recorded using the Royal Horticultural Society (RHS) color chart. The data recorded for all morphological characters was transformed into qualitative data by assigning the respective numerical note or rank based on state of expression of each character mentioned in Table 2. To reveal the morphological similarities and dissimilarities among characterized rose genotypes, recorded morphological data was analyzed and dendrogram was generated (using average linkage between the groups) using hierarchical cluster analysis based on Euclidian distance values with IBM SPSS statistic software (version 20).

Results and Discussion

Vegetative Characteristics

Plant growths, anthocyanin coloration, prickle and leaf characteristics

All vegetative traits used for characterization of germplasm and the number of genotypes identified for each trait was mentioned in Table 2. The genotypes noticed a considerable amount of variability for almost all the parameters. The type of growth observed among the genotypes was shrub, bed, and climber and majority of the germplasm were found with shrub type growth behavior. While the growth habit of studied genotypes varied from upright to spreading. Maximum genotypes were identified with semi-upright and intermediate growth behavior. Plant height in different genotypes varied from tall (>150 cm) to short (<60 cm). The growth type, habit and height of the plants collectively decide the architecture of plants, which is indirectly responsible for gathering resources from the environment. Most commercial varieties belonging to the hybrid tea group were found with a shrub- upright/semi-upright/ intermediate with medium- tall plant height. Whereas floribundas had the characteristic feature of low growing

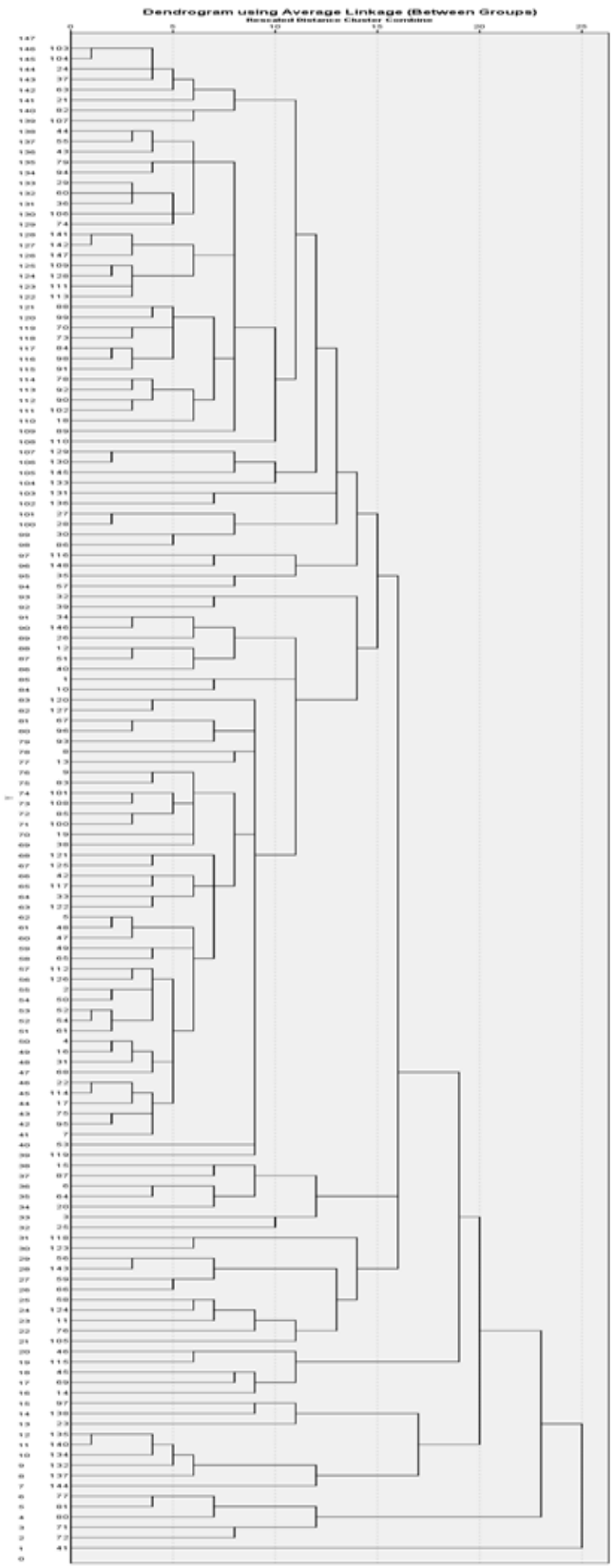

Figure 1: Hierarchical cluster analysis dendrogram of rose genotypes based on morphological data using average linkage (between groups) (Names of the rose genotypes with sequence mentioned in Table)

Table 1: List of characterized rose genotypes

Population	*Rose genotypes*
Modern rose genotypes (128)	Abhisarika, Anurag, Arjun, Arka Parimala, Aruna, Ashwini, Bhim, Century Two seedling, Chambe-di-Kali, Chitra, Dil-Ki-Rani, Dr. B.P. Pal, Dr. Benjamin Pal, Dr. Bharat Ram, Dr. M.S. Randhawa, Dr. R.R. Pal, Dulhan, Eiffel tower × Queen Elizabeth, Ganga, Golden Afternoon, Haseena, Homage, Jawani, Lalima, Lal Makhmal, Madhosh, Maharani, Mother Teresa, Mridula, Mrinalini, Mrs. K. B. Sharma, Nayika, Nehru Centenary, Nurjahan, Pink Montezuma, Preyasi, Priyadharshini, Pusa Ajay, Pusa Arun, Pusa Bahadur, Pusa Garima, Pusa Mansij, Pusa Mohit, Pusa Priya, Pusa Sonara, Raja Ram Mohan Roy, Raja Surendra Singh of Nalagarh, Rajkumari, Raktagandha, Raktima, Ranjana, Ratnaar, Sahasradhara, Shanti Pal, Shreyasi, Sir. C. V. Raman, Soma, Sugandha, Surabhi, Surekha, Surkhab, Jawahar, Shiloz Mukherjee, Indian Princess, Pusa Gaurav, Pusa Shatabdi, Akash Sundari, Delhi White Powder Puff, Delhi Pink Powder Puff, Anitha, Arunima, Banjaran, Chingari, Deepak, Delhi Brightness, Delhi Princess, Dr. S. S. Bhatnagar, Himangini, Jantar Mantar, Krishna, Lahar, Loree, Madhura, Manmatha, Manasi, Mohini, Navneet, Neelambari, Prema, Punchu, Pusa Abhishek, Pusa Barahmasi, Pusa Komal, Pusa Pitambar, Rupali, Sabnam, Sadabahar, Shola, Sindhoor, Suchitra, Surdas, Suryakiran, Suryodaya, Tarang, Pusa Virangana, Pusa Manhar, Pusa Urmil, Pusa Muskan, Rose Sherbet, Velvet Fragrance, Perfume de French Comete, Papa Meilland, Melody Perfume, Elle, Karen Blixon, Christian Dior, Bonne Nuit, Sweet Afton, Oklahoma, Midas Touch, Memorial Day, Fragrant Plum, Fragrant Lace, Eiffel Tower, Double Delight, Century Two, Brandy, Blue Moon
Wild genotypes (20)	*R. dumalis, R. glutinosa, R. tomentosa, R. macrophylla, R. damascena* var. Himroz*, R. damascena* var. Jwala*, R. damascena* var. Rani Sahiba*, R. indica* × *R. nitida, R. indica major, R. bourboniana, R. chinensis viridiflora, R. lutea, R. brunonni, R. wichuriana, R. moschata, R.multiflora,* Dr. Huey (Hybrid *Wichuriana*), *R. slancensis, Rosa spp.(*Nepal*), Rosa spp.* (Kakinada rose)

Table 2: Number of genotypes identified for each characteristic state in the studied rose germplasm

S. No.	*Characteristic*	*State and No of genotypes*	*S. No.*	*Characteristic*	*State and No of genotypes*
1.	Plant growth type	Bed (34), Shrub (110), Climber (4)	25.	Density of petals	Very loose (18), Loose (37), Medium (37), Dense (74)
2.	Plant growth character	Up-right (53), Semi-upright (68), Intermediate (18), Moderately spreading (9), Strongly spreading (0)	26.	Flower shape	Round (110), Irregularly round (12), Star shaped (26)
3.	Plant height	Very short (0), Short (14), Medium (80), Tall (45), Very tall (9)	27.	Flower side view of the upper part	Flat (51), Flatted convex (97), Convex (0)
4.	Young shoot anthocyanin coloration	Present (143), Absent (5)	28.	Flower side view of the lower part	Concave (39), Flat (50), Flatted Convex (37), Convex (22)
5.	Young shoot-intensity of anthocyanin coloration	Very weak (18), Weak (27), Medium (41), Strong (43), Very Strong (13)	29.	Flower fragrance	Absent (22), Weak (48), Medium (37), Strong (41)
6.	Stem-Number of prickles	Absent (6), Few (18), Medium (58), Many (64)	30.	Sepal extensions	Absent (7), Few (89), Medium (41) Many (10)
7.	Leaf Size	Small (7), Medium (89), Large (52)	31.	Reflexing of the petals	Absent (13), Present (134)
8.	Leaf Intensity of green Color on upper side	Very light (0), Light (3), Medium (51), Dark (74), Very dark (20)	32.	Petal shape	Elliptic (3), Obovate (115), Rounded (29)
9.	Leaf anthocyanin Coloration	Absent (129), Present (19)	33.	Petal incisions	Absent (44), Weak (59), Medium (16), Strong (29)
10.	Leaf glossiness on upper side	Absent (14), Weak (42) Medium (58), Strong (38)	34.	Petal reflexing of margin	Absent (16), Weak (37), Medium (57), Strong (37)
11.	Leaflet undulation of margin:	Absent (8), Weak (62), Medium (53), Strong (24)	35.	Petal undulation of margin	Absent (14), Weak (38), Medium (61), Strong (35)
12.	Leaflet serration of margin	Absent (0), Fine (19), Medium (51), Dense (78)	36.	Petal length	Very short (0), Short (9), Medium (94), Long (38), Very long (7)
13.	Terminal leaflet: shape of the blade	Narrow elliptic (14) Medium elliptic (48), Ovate (64), Circular (22)	37.	Petal width	Very short (0), Short (9), Medium (82), Long (47), Very long (9)

14.	Terminal leaflet: Shape of the base of the blade	Acute (27), Obtuse (88) Rounded (32), Cordate (1)	38.	Petal: Number of Colors on inner side	One (134), Two (11), More than two (2)
15.	Terminal leaflet: shape of the apex of the blade	Acuminate (94), Acute (54), Obtuse (0), Round (0)	39.	Varieties with one Color on inner side of the petal	Lighter towards the base (25), Uniform (127), Lighter towards the top (2)
16.	Flowering shoot: Flowering shoot laterals	Present (68), Absent (80)	40.	Petal: Spot at base of inner side	Absent (7), Present (141)
17.	Flowering shoot: Number of flowering laterals	Few (20), Medium (25), Many (23)	41.	Size of the spot at base of inner side	Small (83), Medium (29), Large (29)
18.	Flowering shoot: Number of flowers (varieties with no flowering laterals)	Few (58), Medium (11), Many (10)	42.	Petal: Spot at the base of outer side	Absent (7), Present (141)
19.	Flowering shoot: number of flowers per lateral (varieties with flowering laterals)	Few (18), Medium (25), Many (27)	43.	Size of the spot at base of outer side	Small (83), Medium (23), Large (24)
20.	Flower bud: shape of longitudinal section	Elliptic (10), Round (13), Ovate (88), Broad ovate (24)	44.	Outer stamen predominant Color of the filament	White (1), Green (3), Light yellow (20), Medium yellow (67), Orange (24), Pink (26), Red (4), Brown red (0), Purple (1)
21.	Flower type	Single (9), Semi-double (56), Double (86)	45.	Seed vessel size	Very small (12), Small (71), Medium (60), Large (3), Very large (1)
22.	Flower color group	White or near white (16), White blend (1), Green (1), Yellow (4), Yellow blend (3), Orange (7), Orange blend (4), Pink (67), Pink blend (7), Red (24), Red blend (1), Red Purple (1), Purple (2), Violet blend (2), Brown blend (0), Multi Colored (2), Mauve (2), Apricot blend (6)	46.	Hip: Shape of longitudinal section	Funnel shaped (32), Picture shaped (112), Pear shaped (3)
23.	Flower diameter	Small (33), Medium (44), Large (71)	47.	Flower: Length of pedicel	Short (54), Medium (61), Long (33)
24.	Flower color of the center	Green (0), Yellow (13), Orange (10), Pink (8), Red (4), Purple (2)	48.	Venation of petals	Absent (35), Weak (50), Medium (36), Strong (27)

habit, most of these types had bed type of growth with intermediate/spreading/moderately spreading growing habit. While climbing growth type was observed in very few genotypes, most of which were wild except cultivar Dr. Huey.

Anthocyanin coloration on young shoots and leaves and its hue is another important adaptive feature of roses. Presence of anthocyanin pigment is highly correlated with resistance to various biotic (fungi, herbivores) and abiotic factors (cold, excess radiation both visible as well as UV) in plants (Karageorgou and Manetas, 2006). Nearly, 95% of genotypes found with anthocyanin coloration on young shoots and leaves except for few (Lalima, Nehru Centenary, Pusa Bahadur, Sabnam, *R. lutea*) (Figure 2). Of 143 pigmented varieties, 13, 43 and 41 genotypes had very strong, strong, and medium pigment intensity on young shoots. The genotypes with more color intensity might carry some amount of tolerance against abiotic stress factors. Similarly, prickles provide defense against number of predators, at the same time provide support to the plant. The genotypes with presence of prickles greatly varied in their color as well as in shape. The prickle variability present in the genotypes can be used for breeding genotypes with herbivore tolerance (Gaurav *et al*., 2018). But in modern rose breeding prickle-free character is preferred over prickle character for ease of harvesting and maintenance. Genotypes identified with prickle-free characters were Pusa Mohit, Delhi White Powder Puff, Pusa Komal, Rupali and *R. lutea*.

Leaf size, intensity of green color on the leaves, leaf glossiness, and undulation, serration of leaf margin and shape of the leaflet were observed. The variability between the wild and cultivated types was observed for leaf size, leaflet size, and number. In modern cultivars leaves were found with medium to large leaf size with maximum 5 leaflets, whereas in wild types leaf size was comparatively small, and the number of leaflets varied from 5 to 9. Likewise, intensity of green color and glossiness of the leaves also

Figure 2: Rose genotypes devoid of anthocyanin pigmentation of young shoots.

Figure 3: Rose genotypes having more than one petal Color.

varied among modern roses, but in wild genotypes, glossiness and green color is absent in most of them. The differences noticed for leaf characters among wild and cultivated types might be due to the involvement of artificial

Figure 4: Rose genotypes identified with more number of flowering laterals

selection of genotypes for attractive leaf characteristics over the generations. Many of the characterized genotypes were found with medium to large size (10–16 cm). While few genotypes, such as Dil-ki-Rani, Delhi White Powder Puff, Arunima, Mansi, *R. slancensis*, *R. lutea*, Dr. Huey were observed with small leaf and leaflet sizes. Genotypes were identified with fine medium and dense leaf serration. Instead of acting as a mere marker, leaf serration is found to be an important adaptable trait for plants growing in temperate regions. Leaves with teethed margins have better transpiration and photosynthetic abilities than leaves with smooth margins (Steve Nix, 2018). Leaf undulation is another marker for studying phylogeny. The trait improves the contact between cells which allows efficient signaling, increases epidermal integrity, helps in leaf flex and alleviates the stress caused by their own growth (Sotiriou *et al*., 2018). More than 94% of the characterized roses were found with varying degrees of undulation.

Flowering Characteristics

Flowering shoot and flowering shoot laterals

The modern grouping system of roses indirectly depends on flower bearing nature of the plant along with flower type

and plant growth behavior. Hybrid Tea varieties had mainly upright growth habit with around 1 to 2 m plant height and bears mostly single, well-shaped flowers with high spiraling centers. Floribundas, another class, produce abundant clusters of small flowers on its flowering stems. Majority of hybrid tea genotypes perfectly fit into its characteristic nature with 'single flowering shoot or absence of flowering laterals', while few hybrid tea cultivars varied slightly with this character by bearing few or medium number of laterals on their main flowering shoots. Although these varieties were identified with flowering laterals, the flower and plant growth behaviour characteristics match with the hybrid tea group. Likewise, almost all floribunda varieties observed in the study were found with varying number of flowering laterals (Figure 4) on their flowering shoots except nine cultivars. However, these varieties were grouped under the category of floribundas due to their lower plant growth habit and flower characteristic resemblance with floribunda types. Whereas in wild species, flower laterals were present in all apart from Kakinada rose and *R. indica major.*

Flower bud shape, flower type, size, flower color, petal density and fragrance

Flower bud shapes in majority of genotypes were ovate and broadly ovate. Very few genotypes had elliptic and round bud shape. Petal number decides the flower type in roses. Usually, wild rose species have a 'simple flower' with typically five petals per flower, while in modern roses, flowers are found to be double type consisting of >10 petals per flower (Panwar *et al*., 2012). Among wild genotypes single flowers were found in *R. slancensis*, *R. brunonni*, *R. macrophylla*, *R. wichuriana*, *R. moschata*, *R. multiflora*, *R. glutinosa*, *Rosa* spp. (Nepal species) and *R. tomentosa* and remaining few genotypes were identified with semi-double and double flowers. The semi double wild genotypes present in the study were *R. indica* × *R. nitida*, *R. indica major*, *R. dumalis*, *R. lutea*, *R. bourboniana* and Dr. Huey. Whereas in modern roses, 86 double, 53 semi-double and 9 single type flowers were identified. Flower color is foremost important feature of roses. Around, 16 different flower color groups (as per DUS characteristic groups) were identified in the study (Table 2 and Figure 3). Flower size in roses varies from 1.25 (miniatures) to 17.5 cm (Hybrid Teas). Diameter of the flower depends upon size of the flower organs, especially petals. The flower size varies from 3.5 to 12 cm among the studied genotypes.

Petal density depends upon petal number, arrangement and flower size. Majority of the genotypes with double flowers were identified under the dense petal group. Likewise, flower type with inward petal arrangements such as blooms with quartered, cupped, globular, rosette and high centered blooms with many petals showed more petal density than exerted flat bloom shapes. The shape of rose blooms depends upon the size of the petals (inner and outer whorl of the flower), petal undulation, serration, and refluxing of petal margins. In different roses, the bloom shape varies from round, irregular and star. Among the studied genotypes 74% of the flowers possess round flower shape, while 17 and 8% flowers possess star and irregular flower shape. The star shaped flowers were found with strong petal reflexion. Likewise, serration and undulation were more common in genotypes with round and irregularly round flowers. In bloom types such as cup, pompon and in globular shaped flowers petal reflexion is comparatively poor or absent as compared to the flowers with flat, rosette and quartered shape. Flower fragrance is another superior economic trait. The quality, quantity, and composition of the hundreds of volatile molecules decide the scent profile (Prasad *et al*., 2006; Aparna *et al*., 2019). A great amount of diversity is noticed for fragrance trait in studied roses. In this study, around 41 highly fragrant, 37 medium and 48 weak fragrance types were identified and 22 were found to be non-fragrant.

Petal basal spot, venation, filament color, sepal extensions, seed vessel and petal venation

Based on presence and absence of basal spot genotypes were grouped into two. Basal spot was absent in very few varieties. In majority of the genotypes color of basal spot is yellow. color of the basal spot was indistinguishable if color of the petal is more intense. Likewise, flowers with yellow secondary color distribution on petal base (Banjaran, Chingari) were not seen with petal spot. Similarly, genotypes had flowers with uniform color distribution across petal with minute petal attachment (Himangini, Loree and Fragrant Lace) were not seen with petal spot. Strong, medium to weak petal venation was observed in 18 and 58% of genotypes, while clear venation was not seen in 28%. Flowers having intense color and petal thickness were seen with unclear venation. Color of the filament is concerned, nearly 73% of the studied genotypes were found with yellow filament color. But in few genotypes, filament color was slightly influenced by petal color. Certain degree of similarity was observed with outer stamen filament color with petal color in genotypes such as Aruna, Dr. Bharatram, Ashwini, century two seedling, Dil-ki-Rani etc. filament colors of these genotypes varied with dark colors like orange, pink, red and purple. But in few genotypes (Oklahoma, *R. damascena* cv. Ranisahiba, *R. lutea*, Fragrant Lace), the demarcation of filaments was prominent with petals and in these types color of the filaments was varied from white to green. Based on sepal extensions genotypes were categorized into four groups (Table 2). Five categories of seed vessels (very small, small, medium, large and very large) were observed in characterized germplasm. The majority (88%) fall in the category of small and medium. Very large hips were observed in varieties 'M. S. Randhawa and Dr. Benjamin Pal. The shape of the hip in majority (75%) types were pitcher,

followed by funnel shape in 21% germplasm and very few genotypes (2%) Chambe-di-kali, Sadabahar and Chingari were found with pear shaped seed vessels. Length of the flower pedicel and its strength along with flowering stem decides the commercial use and quality of flowers. Almost all the genotypes present in hybrid tea group were found with long to medium pedicel length, whereas most of floribundas and wild roses were found with small pedicel length.

Cluster analysis among rose genotypes based on observed dus characteristics

Hierarchical cluster analysis separated the genotypes into eight different clusters (Figure 1). Cluster-I was found with majority of the characterized genotypes (109); which was followed by cluster-III (11), cluster-II and VIII (7 each), cluster-IV & VII (5 each), cluster-V (3) and cluster-VI (1). Within clusters, similarities were observed among the genotypes irrespective of its group (HTs/Floribundas), origin (Exotic/ Indian) and habitat (wild/domesticated). For example, cluster-I was found with majority of the hybrid teas, floribundas along with 11 wild species. Cluster-II was found with cultivars Arjun, Ashwini, Dr. M.S. Randhawa, Golden Afternoon, Madhosh, Jawani, and Neelambari. Among these varieties morphological uniformity was observed for around 9 traits. Cluster-III was identified with cultivars, Dil-ki-Rani, Sir C. V. Raman, Sugandha, Surabhi, Akash Sundari, Dr. S. S. Bhatnagar, Tarang, Fragrant Lace, Midas Touch, Oklahoma, *R. bourboniana*. For these genotypes, complete trait uniformity observed for around 6 qualitative traits. Similarly, 12 traits were uniform for genotypes present in cluster-IV.

Genotypes Lalima, Sabnam, *R. lutea* were found together in cluster V showed homogeneity for17 DUS characteristics. Cultivar 'Pusa Mansij' is a multi-Colored Hybrid Tea, found alone in cluster-VI, exhibited morphological dissimilarity maximum genotypes with its unique traits like presence of more than two Colors on petals, absence of prickle on immature shoots, absence of petal reflexing, absence of petal margin reflexion, *etc*. Five floribunda varieties Banjaran, Chingari, Loree, Himangini and Lahar were grouped together in major cluster-VII. Among this, high uniformity was observed for majority of qualitative and pseudo-qualitative characters along with few quantitative characters such as leaf size, flower type, size of the basal spots. Cluster-VIII was found with all six wild genotypes *R. wichuriana*, *R. moschata*, *R. multiflora*, *R. glutinosa*, *Rosa* spp. (Nepal species) and *R. chinensis viridiflora* among these species complete morphological similarity was observed for maximum number of quantitative features (flower diameter, sepal extensions, seed vessel size, length of the flower pedicel, petal undulation and reflexing) along with qualitative and pseudo-qualitative traits.

The present study revealed the vast variability within characterized rose genotypes, including wild species for various vegetative and flowering traits. Characterized data of different traits could be used as reference collection for the identification of rose varieties. The information on different traits also helpful for future improvement programmes for developing more competent types. The given information also helps the breeders and nurserymen seek the protection of their new material under PPV and FRA, New Delhi.

References

Aparna V, K V Bhat, T Janakiram, K V Prasad, D V S Raju, C Bharadwaj, S V Amitha Mithra, Namita, K P Singh, and P Sapna (2020) Characterization of Indian bred rose varieties using morphological and molecular markers for conservation and sustainable management. *Physiol. Mol. Biol. Plants* **26:** 95-106.

Aparna V, K V Bhat, T Janakiram, K V Prasad, D V S Raju, P Sapna, Namita and K P Singh (2019) Assessment of genetic diversity and population structure of fragrant rose (*Rosa* × *hybrida* L.) varieties using microsatellite markers. *Indian J. Agril. Sci.* **89 (11):** 1964-70.

Gaurav AK, Namita, DVS Raju, M Singh, S Panwar and KG Vani (2018) Morphological Assessment of Rose species and variants using multivariate analysis. *Int. J. Chem. Stud.* **6(2):** 3277-3282.

Karageorgou P and Y Manetas (2006) The importance of being red when young: anthocyanin and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. *Tree Physiol.* **26:** 613-621.

Panwar S, K P Singh and Namita (2012) Assessment of variability, heritable components and grouping of Indian rose (*Rosa* × *hybrida* L.) genotypes based on DUS guidelines. *Indian J. Agril. Sci.* **82(10):** 875-880.

Panwar S, KP Singh, Namita and H Sonah (2010) Genetic divergence analysis in rose (*Rosa* x *hybrida*) using morphological markers. *J. Ornam. Hortic.* **13(2):** 122-126.

PPV&FRA (2009) guidelines for the conduct of test for distinctiveness, uniformity and stability on rose, government of India, New Delhi.

Prasad K V, S Kumar and M L Choudhary (2006) Molecular characterization of fragrant rose varieties. *Indian J. Hortic.* **63:** 229-234.

Sotiriou P, E Giannoutsou, E Panteris, B Galatis and P Apostolakos (2018) Local differentiation of cell wall matrix polysaccharides in sinuous pavement cells: it's possible involvement in the flexibility of cell shape. *Plant Biol.* **20:** 223- 237.

Steve Nix (2018) Tree leaf key: leaf margin serrated versus smooth *(https://www.thoughtco.com).*

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2023.00036.2.07

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

NBPGR-PDS: A Precision Tool for Plant Germplasm Collecting

Ankur Tomar, Anjula Pandey, KC Bhatt*, NS Panwar and SP Ahlawat

Abstract

An explorer uses a passport datasheet to record information of collected germplasm in the field. The present paper discusses the utility of a Digital Passport Data Sheet (NBPGR-PDS) for collecting information during exploration. NBPGR-PDS is an App developed for Android users that supports Android version (4.1; API 16) to version (10.0; API 30) smartphones with offline inbuilt SQLite DB, Core Java program code in Android Studio Platform. App containing PDS is a digital book to collect large amounts of data with photographs to be stored systematically. Users can easily transfer their data in MS Excel format. The App also has the facility to operate offline and standalone to protect data at the user's end and can be freely downloaded from Google Play Store (https://play.google.com/store/apps/details?id=com.nbpgr.nbpgr_pds) for researchers, students, botanists and entrepreneurs.. It has added features like 'More Info' and 'Feedback', which the users can easily access to additional details and share queries directly to the host Institute. This paper includes details on developing the App for germplasm collectors, growers, and researchers to maintain records of collected germplasm in digital form.

Keywords: Android, SQLite DB, Germplasm, SDK, Modern tool.

ICAR-National Bureau of Plant Genetic Resources, New Delhi, India.

***Author for correspondence:**

kailash.bhatt@icar.gov.in

Received: 15/07/2022 **Revised:** 08/02/2023

Accepted: 10/02/2023

How to cite this article: Tomar, A., Pandey, A., Bhatt, K.C., Panwar, N.S., Ahlawat, S.P. (2023). NBPGR-PDS: A Precision Tool for Plant Exploration and Germplasm Collection. *Indian J. Plant Genetic Resources*. 36(2), 250-255. **DOI:** 10.5958/0976-1926.2023.00036.2.07

Introduction

The main objective of collecting is to capture available genetic diversity for *ex-situ* conservation and document the genetic diversity of the target species in a particular area. Passport data of existing collections are an important source of information since they help to establish the historical occurrence of the species in particular areas and the sampling it has been subjected to in the past. In Plant Genetic Resources science (PGR), documentation fundamentally refers to recording, organizing and analyzing information to understand the germplasm and make management decisions. Usually, while on exploration, the plant collector records information on details of many passport data of the germplasm acquisitions (domestic collections and exotic introductions). The shape of the data in hard format may be a book form containing sheets (called passport datasheets). As per Hawkes (1976), the passport data should be recorded on pre-numbered sheets. A comprehensive review of data recording in the field is also provided by Moss and Guarino (1995). However, the information on both the essential and optional fields needs to be recorded in the passport datasheet at the site of the collection (Zippel *et al.* 2010).

Documentation in most of the early plant germplasm collection missions was in the form of handwritten records before the "computer era" where passport data were

generally limited to a few records in a field notebook. Exact location and the "unique" number (passport number that identifies the collected germplasm) are supposed to be the most important item in passport data because only this unique identifier number/code of a collected population remains associated with the sample at all times. The passport data book contains various fields/parameters for gathering the observations like species name, country/state/district/village of origin, vernacular/cultivar name, date of collection, collector's number; geographical location (latitude, longitude and altitude), etc. are the major fields for recording the information.

The passport datasheet consists of the following four major parameters:

- **Taxonomic:** Genus, species, subspecies, variety name, pedigree and biological status of sample.
- **Geographical:** Latitude, longitude, altitude, country of origin, state/province and location.
- **Ecological:** Site environment, soil characteristics, temperature and vegetation type.
- **Ethnobotanical:** Local use, cultivation methods and processing of plant parts.

Management of field records in paper bounded passport data book is a cumbersome method, time-consuming and has more scope for inaccuracy (appendix I-II). During survey and germplasm collecting, the explorer is involved in activities like interaction with the farmer (to obtain information about local names, origin and use of the material), data recording, collecting and leveling of samples, taking photographs, gathering and pressing herbarium specimens, etc. NBPGR-Passport Datasheet (PDS) as an App would be the most useful tool for users to manage germplasm collecting records systematically and access them anytime in android smartphones or in app or MS Excel format. Android smartphones are popular communication tools in World Wide Web (WWW) which made possible to generate customized digital applications for the target customers/users. The development of mobile apps in PGR Informatics facilitates enhanced access to PGR information, leading to enhanced utilization. NBPGR so far has developed only two mobile apps "Genebank" and "PGR Map" first of their kind for any genebank in the world and have been developed for both android and iOS (Archak and Agrawal, 2012). The NBPGR-PDS App developed in android studio, is the official integrated development environment (IDE) for android app that can create a unified environment for building the apps for android phones, tablets, android wear, android TV, android auto, etc. Apart from this, structured code modules divide project into units of functionality that can independently be built, tested, and debug.

The present paper has been aimed with the following objective:

- To enhance the pace of recording of data during germplasm collecting along with live location data and camera functionality which will enable to increase the size of field records digitally with perfection.
- To strengthen field-based research using quality information, including identification of unique material and its recorded use for PGR management.
- To manage data digitally in an organized manner and to focus on other relevant areas to enhance their knowledge and to lead exploration activity with innovation.
- Ease of handling and minimal user input.

Materials and Methods

The app is devised by following the android studio application framework, an open source software. Android app contains build. Gradle module where dependencies are synchronized and whole android programs which are dependent on xml and Java files, called activity. Multiple activities were created for main screens as well as menu layouts. Default permissions, *"android.permission.INTERNET"* and *"android.permission.SEND_SMS"* had enabled in the Android Manifest.xml file of the application, which users need to allow these permissions for using *Feedback* options. The first author already complied one of the android applications on same platform namely 'Orchids farming' (Tomar *et al.*, 2019) that is user information given application and runs successfully. Application is freely available on Google Play Store (https://play.google.com/store/apps/details?id=com.nbpgr.nbpgr_pds) with the size is 9.4 MB after installation and will work on any device without acquiring much space. Its space-friendly approach, with the insertion of records, data accumulation capacity of app is increased automatically. "Easy to access" theme adopted in the app ensures secure reliability to users.

Inserting, updating, deleting of records are the important features of the android app devised. The architecture which describes the functionality of the app in a stepwise manner is depicted in Figure 1. Currently, the cumulative

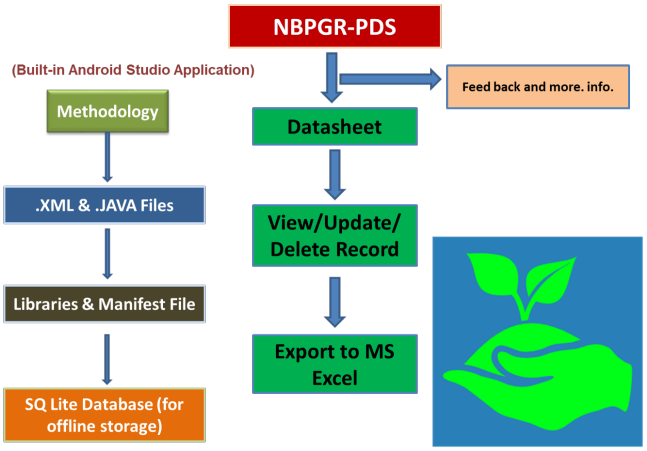

Fig.1: Architecture of app

Table 1: Technical details

Operating system	Android (Linux kernel)
Platform	Google (Android software development kit (SDK))
Programming language	Android Core Java
Software	Integrated development environment(IDE) Android Studio 4.0 version using the Android Development Tools (ADT)
Database	SQLite DB
Package manager	Android Package Kit (APK)
Version	4.1 (Jelly Bean) to 10.0 (Android 10)
Source model	Android is open source
Permission file	Manifest.xml & Build.gradle
File format	.JAVA & Extensible Markup Language (XML)
Availability	https://play.google.com/store/apps/details?id=com.nbpgr.nbpgr_pds

distribution of NBPGR-PDS is 99.8% (Figure 2) as compared to all Android devices belonging to Android version 4.1 (Jelly bean) to version 10.0 (Android 10) smartphones using offline inbuilt SQLite DB, Core Java program code in android studio platform, which generates .apk (android package kit) file for installation as per the requirement of the device. The technical details of app, which work in the integrated environment of android SDK are given in Table 1.

Application contains 20 *AutoCompleteTextView* format fields where users have option to choose or feed the information recorded in selected crop/germplasm (Table 2). There are 16 *EditText* format fields where the user must fill the column as text/number and 4 Searchable Spinner format fields to choose data from the list. The mentioned file format will help the experienced explorers and support new explorers/researchers. Moreover, these format fields will also be helpful in learning the technique of filling passport datasheets efficiently. Major advantages of the app are to get current GPS location of the collecting site with a single click on a button and choose high-definition images of a desired object like whole plant, flower, and field view from a gallery/click live image that would enhance the reliability of the app among the explorers/researchers. The inserted records in SQLite DB can be retrieved by using '*Save in Excel*' Button. This will also convert the .sqlitedb file format record into .xlsx file format and save in mobile internal storage. This is the best way to retrieve inserted information by the explorer from app to the computer system.

Results and Discussion

Devising an app for recording data in digital format has addressed many inherent problems like manual recording leading to mistakes and loss of data using paper field books.

Figure 2: Application Programming Interface (API) level chart

Table 2: Format type and major fields in app

Format type	*Fields*
ScrollView, LinearLayout, RelativeLayout	Layout design
TextView	Heading, Titles,
Button	GetDate, Submit, Get Location, Photograph, Update, Delete
EditText	Date, Collector's No., Accession No., Crop Name, Botanical Name, Crop/ Vernacular Name, Cultivar Name, Village/Block, Region Explored, Longitude, Latitude, Farmer's/ Donor's Name, Ethnic group, Address and Mobile No., Additional Info.
SearchableSpinner	Common Name (searchable-dropdown list), Botanical Name, State, District
AutoCompleteTextView	Collection Site, Biological Status, Frequency, Material, Breeding System, Sample Type, Sample Method, Habitat, Disease Symptoms, Insect infection, Cultural Practices, Season, Associated Crop, Soil Colour, Soil Texture, Topography, Agronomic Scope, Part, Kind, Informant
ImageView	View Image
ListView	List the records in a row
View	Spacing

NBPGR-PDS has almost all source code areas, which hcan be accessed freely and offline manner so that developers can further customize the app to meet specific data collection requirements.

Similarly, first author has designed and developed a similar android-based application called 'Orchidopedia' used for the identification of orchid species with their scientific details and high-quality pictures (Tomar *et al.,* 2022) and working in an offline manner for tribal orchid growers/ farmers. However, NBPGR-PDS is the first of its kind that

Figure 3: Splash screen and demo activity

Figure 4: Splash screen and demo activity

can record crop diversity data and store it systematically. This could be helpful in the planning of future collection missions. It also facilitates easy and rapid data insertion in SQLite Database with low space, can easily viewed/ downloaded using "View Record" option. Data can be exported in "Table format" using the traditional spreadsheet format with a list of entries in rows and corresponding traits in columns. Image file path also stored with individual entry in the database to identify the location. This ability to keep data organized in digital form allows explorers to focus on other tasks simultaneously. In the miscellaneous function, the right side popup menu option can redirect the user on any activity page, showing app flexibility. "How to use" option in the menu popup bar explains the app by showing a small demo to the user. 'Share' option helps users to spread the application in the fastest mode. "More Info"

Figure 5: Recorded activity

option button provides the details on source and contact information, whereas "Feedback" button allows users to interact with the developer of app through inbuilt e-mail address. The data accumulation capacity of app increased automatically with the insertion of records. NBPGR-PDS App allows users to feed information in the inbuilt database as per requirement in simple and straightforward ways hence under mentioned 6 steps for functionality is essential to follow:

1. When app starts working, splash screen will appear (Figure 3), while only demo screen will appear once to show the brief intro of app.
2. After the completion of demo session, screen containing datasheet, and inserted records, more info will be displayed on it, while the feedback option with a menu option will appear on top right corner. While clicking on the datasheet option, user can see 2 scroll view areas. In the first scroll view area, user can insert records using hide and unhide button options, whereas in the second scroll view area, user have the option to check the inserted records as well as to select the updation and deletion options (Figure 4).
3. Users can click the inserted records option to see the number of entries in stack format with the option of export in MS Excel or downloading the data in internal storage derive (Figure 5).
4. Users can easily hide/unhide data as per their need. This functionality gives vital help while accessing particular species detail.
5. 'Feedback' option: e-mail service helps explorers to communicate with in easy manner (Figure 6).
6. 'More Info' option gives details about the organisation and their developer info which will assist the user in performing the desired task smoothly.

Figure 6: Feedback activity and more info. activity

Conclusion

Earlier, the documentation of information pertaining to plant explorations in field began with the handwritten data and later using cyclostyled sheets to be filled by explorers in field. Later, improved versions contained printed passport data sheets with optional records that were developed in early 1990s and now it has taken shape of digital format as "PDS-app". The digital format of NBPGR-PDS was devised to facilitate the task of keeping digital record of passport data of collected germplasm which is fundamental to use in plant breeding and varietal development. The PDS-App was utilized to record the locality information (latitudes, longitude and altitude) without any hindrance in its functionality.

The app has been validated during the execution of national exploration programmes in different states namely Chhattisgarh, Maharashtra, Uttarakhand and Sikkim during the year 2021-22 and 2022-23 for collecting various agricultural/horticultural crops/species like minor fruits-chironji (*Buchanania lanzan*), field crops (rice, maize, wheat, vegetables) and medicinal and aromatic plants from cultivated land to deep forest areas/localities like farmers' field, wastelands, high lands, wild habitats, forest areas, etc. The outcome from the testing phase at mentioned regions are minor technical bugs/errors, altitude fetching errors and repeatedly typing of collector's no. in a predefined format. Including waypoints for generating route map in the revised version will facilitate the opportunity to revisit the already explored area and guide in planning future explorations. We enabled the voice typing technique only in the feedback section for trial because we already cleared that the application supports multi-verse smartphone devices; therefore, voice typing is not supportive to all versions of smartphones. Feedback from readers is welcomed for improvement of this app and suggestions for betterment.

After use of the app by the explorer/researchers during exploration, the following points were noted:

- Totally user-friendly, operative in smartphones, and available round the clock.
- Use in offline mode, does not require internet facility/ connectivity.
- Strong and reliable substitute for paper bounded passport data.
- Efficient in recording data in field and easily accessible from anywhere and anytime.
- Facility to accommodate large data and addition and deletion of fields.
- Operative in English language but has scope for other languages.
- Managing vast inserted records by transferring SQLite DB records to Excel format by easy click.
- Users are expected to provide feedback for its efficiency and improvement of this tool in future.

Acknowledgement

Authors are thankful to the Director, ICAR-NBPGR, New Delhi for support in developing the app and users/explorers for encouraging to devise a digital format of passport data recording. Thanks are also due to various explorers and users for providing feedback on the application.

References

Archak, S. & Agrawal, R.C. (2013). PGR informatics at the National Bureau of Plant Genetic Resources: status, challenges and future chapter 13. In: Halewood, M., Brahmi, P., Mathur, P.N. and Bansal, K.C. Editors. (2013) *A road map for implementing the multilateral system of access and benefit-sharing in India*. Bioversity International, Rome; ICAR and NBPGR, New Delhi, 107-112.

Hawkes, J.G. (1976). Manual for Field Collectors (Seed Crops). *International Board for Plant Genetic Resources*, FAO, Rome, Italy, 36.

Moss, H. & Guarino, L. (1995). Gathering and recording data in the field. In: Collecting plant genetic diversity: technical guidelines (eds. Guarino, L., Ramanatha Rao, V. and Reid, R.). CAB International, Wallingford, 367-417.

Tomar Ankur, Pamarthi, R.K., De, L.C., Rampal, Singh, R.K. & Singh, D.R. (2019). Mobile App - Android Application on "Orchid Farming" based on North Eastern States of India. *Indian J Hortic.* **76(4):** 752-756.

Tomar Ankur, R.K., Pamarthi, R.K., Rampal, De, L.C., Biswas, S.S. & Singh, D.R. (2022). Orchidopedia App -A Tool for Exploration and Collection of Orchid Species. *Indian J. Plant Genet. Resour.*, **35(1):** 73-79.

Zippel E, Wilhalm T. & Thiel-Egenter, C. (2010). Manual on vascular plant recording techniques in the field and protocols for ATBI + M sites – inventory and sampling of specimens, chapter 14. In: Eymann J, Degreef J, HäuserCh, Monje JC, Samyn Y, VandenSpiegel D (eds) Manual on field recording techniques and protocols for all taxa biodiversity inventories and monitoring, **8(2):** 346-376.

Appendix-I

ICAR-National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012

PASSPORT DATA FORM

Collector's Name and Address:
Collaborating Institute: Name of Scientist(s) and Address:
Area Explored:
Duration of Exploration: From…….. To:……..

Sr. No.	Collector No.	IC No.	Crop's common name	Botanical name	Cultivar/ Landrace name	Biological status	Type of material	Collection date	Collecting site /acquisition source	Frequency
1.										
2.										

Sr. No.	Collector No.	Sample type	Sampling method	Habitat	Site of collection				Latitude (N)	Longitude (E)	Altitude (m)	Ethnobotanical information/ Ethnic group	Remarks (Trait-specific characters)
					Village	Mandal/Taluk/ Tehsil	District	State					
1.													
2.													

For allotting IC number the completed sheets should be sent along with samples (2000/4000 seeds of self/cross pollinated crops) to: **The Head, Division of Plant Exploration and Germplasm Collection, ICAR- National Bureau of Plant Genetic Resources (old building), Pusa Campus, New Delhi-110 012.**
E mail address: NBPGR.exploration@icar.gov.in (please also send soft copy of passport data in MS Excel through e mail), Phone: 011-2584 8405 (O)
For taking IC number to the vegetatively propagated crops/species, **also furnish the certificate of conservation and maintaining in field gene bank/NAGs**

Useful tips

Collector number: denotes a unique/primary identity assigned by the collector at the time of collection, given in abbreviated form of collectors name followed by accession number (for example: KCB/KP/231 (expanded form is: collector number assigned in an exploration by KC Bhatt as team leader and K Pradheep as collaborator/associate; germplasm sample sequence/ serial number is 231)
Biological status: (***Wild***-All wild species that are related and part of the genepool from which genetic introgression into cultivated species is possible using conventional methods; ***Weedy***-Weedy form of cultivated species occurring in companionship (fields) of some other cultivated species; ***Landrace/Traditional cultivar/ Primitive cultivars/ Farmers variety***- All cultigens under cultivation in farmer's field with/ without specific names frequently associated with unique traits identified by farmers; ***Breeding line***-Semi-finished products or segregating material generated out of hybridization programme to meet specific breeding objectives; ***Elite line/Advanced/ Improved cultivar***- Selection from population, from coordinated trial (AVTII line), improved cultigens of common knowledge in commercial cultivation (extent, released by institution/organization/State) but not notified from the Central Sub-Committee on crop standards, Notification and Release of Varieties of Agricultural and Horticultural Crops and Parental lines of hybrids; ***Released cultivar/ Hybrid***-Varieties/hybrids notified and released by the Central Sub-Committee on crop standards, Notification and Release of Varieties of Agricultural and Horticultural Crops; ***Genetic stock/ Registered germplasm***- Trait and gene specific germplasm experimentally developed/identified through scientific interventions (e.g. sources of resistance, mutant, cytogenetic stock etc.) which is registered for unique trait(s) at ICAR-NBPGR; ***Others***-Doubtful or material with unknown biological status)
Type of material: Seed/fruit/inflorescence/root/underground parts/cuttings/live plants
Collecting site/acquisition source: Farmer's field/ threshing yard/ fallow/ farm store/ wild/ orchard/home garden/ market/ aquatic/Institute name (if others, give source name)
Frequency: Abundant/frequent/occasional/rare; **Sample type:** Population/pure line/Individual; **Sampling method:** Bulk/random/non-random/individual plant
Habitat: Cultivated/disturbed/partially disturbed/rangeland/forest/aquatic habitat. Fill the **latitude and longitude** in decimal points (ex. 75.23) instead of degree and minutes (75^0 23').
Note: Please do not merge, delete, change sequence and content of columns in table. However, append additional rows as per need (number of accessions) in above table. Submit separate datasheet for explorations conducted by different collectors or at varying period. For breeding line/genetic stock developed in Institute, location of farm/institute to be filled.

Source: http://www.nbpgr.ernet.in/Downloads/cid/1012.aspx

Appendix-II

Date……Collector's No……Accession No……	
Botanical Name…… Common Name (English)…… Crop/Vern.	
Name…… Cultivar name…… Region Explored……	
Village/Block……District……State…… Latitude……^{0}N	
Longitude……^{0}E Altitude……m	
Temp…… Rainfall ……	
COLLECTION SITE	1. Natural wild 2.Disturbed wild 3.Farmer's field 4.Threshing yard 5.Fallow 6.Farm store 7.Market 8.Garden 9.Institute 10……
BIOLOGICAL STATUS	1.Wild 2.Weed 3.Landrace 4.Primitive cultivar 5. Breeder's line
FREQUENCY	1.Abundant 2.Frequent 3.Occassional 4.Rare
MATERIAL	1. Seeds 2.Fruits 3.Inflorescence 4.Roots 5.Tubers 6. Rhizomes 7. Suckers 8.Live plants 9.Herbarium 10……
BREEDING SYSTEM	1.Self-pollinated 2.Cross-pollinated 3.Vegetatively propagated
SAMPLE TYPE	1.Population 2.Pure line 3.Individual plant
SAMPLE METHOD	1.Bulk 2.Random 3.Selective (non-random)
HABITAT	1. Cultivated 2.Disturbed 3.Partly disturbed 4.Rangeland 5……
DISEASE SYMPTOMS	1.Susceptible 2.Mildly susceptible 3.Tolerant 4.Resistant 5.Immune
INSECT/ PEST/ NEMATODE INFECTION	1. Mild 2.Moderate 3. High
CULTURAL PRACTICE	1. Irrigated 2.Rainfed 3.Arid 4.Wet 5……
SEASON	1.Kharif 2.Rabi 3.Spring-summer 4.Perennial type
ASSOCIATED FLORA	1. Sole 2.Mixed with……
SOIL COLOUR	1. Black 2. Yellow 3.Red 4.Brown 5……
SOIL TEXTURE	1.Sandy 2.Sandy loam 3.Loam 4.Silt loam 5.Clay 6.Silt
TOPOGRAPHY	1.Swamp 2.Flood plain 3.Level 4.Undulating 5.Hilly dissected 6.Steeply dissected 7.Mountainous 8.Valley
AGRONOMIC SCORE	1.Very poor 2. Poor 3. Average 4. Good 5. Very good
ETHNOBOTANICAL USES	
PART(S)	1. Stem 2. Leaf 3.Root 4. Fruit 5.Flower 6.Whole plant 7.Seed 8.Others
KIND	1.Food 2.Medicine 3.Fibre 4.Timber 5.Fodder 6.Fuel 7.Insecticide/ Pesticide 8.Others
HOW USED	……
INFORMANT(S)	1.Local Vaidya 2.Housewife 3.Old folk 4.Graziers /Shepherds 5.Others
PHOTOGRAPH	1.Colour/Video
FARMER'S/ DONOR'S NAME……ETHNIC GROUP……Mobile No……	
ADDRESS	
PLANT CHARACTERISTICS/ USES ADDL. NOTES	

Source: http://www.nbpgr.ernet.in/Downloads/cid/1012.aspx

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2023.00036.2.08

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Breeding for Higher Ascorbic Acid and Mineral Nutrients in Snowball Cauliflower (*Brassica oleracea* var. *botrytis* L.)

Hanuman Ram[1], Amish K. Sureja[2*], Shyam S. Dey[2] and Abhijit Kar[3]

Abstract

The components of genetic variance and gene action effects for ascorbic acid and mineral nutrient contents in snowball cauliflower lines were estimated through line × tester analysis involving 5 Ogura based CMS lines and 7 male fertile testers. The mean squares due to lines and testers were significant for ascorbic acid and mineral nutrients (Ca, Mg, Na, K, S, Fe, Mn and Zn). Ogu 103 was best performing CMS line for K (182.32 mg/100 g), S (29.48 mg/100 g), Ca (23.19 mg/100 g), Mg (13.22 mg/100 g), Zn (313.33 µg/100 g) and Mn (235.00 µg/100 g) whereas maximum ascorbic acid (81.26), Na (24.37) and Fe content (645.03 µg/100 g) was noted in Ogu 119, Ogu 13-85 and Ogu 13-01, respectively. Among the testers, Kt-18 recorded maximum value (mg/100 g) for Na (98.03), K (277.52), S (42.87), Ca (43.73) and Mg (29.44), respectively, whereas Kt-22 had highest ascorbic acid (70.84 mg/100 g) and Mn content (343.50 µg/100 g) and Sel-26 had maximum Fe (1424.93 µg/100 g) and Zn (458.70 µg/100 g) contents. The best-performing hybrids for different macro-nutrients (mg/100 g) were, Ogu 101 × Sel-26 for ascorbic acid (94.05), Ogu 103 × Sel-26 for Na (98.63), Ogu 13-01 × Sel-26 for K (300.18), Ogu 101 × Lalchowk Maghi for S (49.34), Ogu 119 × Suprimax Late for Ca (54.65), Ogu 13-85 × DB-187 for Mg (35.60). For micronutrients (µg/100 g FW), the best hybrids were Ogu 13-01 × DB-187 for Fe (1775.60), Ogu 101 × Lalchowk Maghi for Zn (642.10) and Ogu 101 × Kt-22 for Mn content (381.60). Predominance of dominance component of variance was observed for vitamin C and mineral nutrients. The traits studied had narrow differences among GCV, PCV and broad sense heritability values, suggesting low effect of environment. The variance due to general (σ^2gca) and specific combining abilities (σ^2sca) were highly significant indicating the importance of both additive (σ^2A) as well as non-additive (σ^2D) type of gene actions. However, the ratios of σ^2gca/ σ^2sca (<1) and σ^2A/σ^2D (<1) revealed the preponderance of non-additive variance for the inheritance of traits studied. The study's results suggest the possibility of improving these traits through recurrent selection and hybridization.

Keywords: Cauliflower, Cytoplasmic male sterility, Gene action, Line × tester, Minerals.

[1]Division of Crop Improvement, ICAR-CIAH, Bikaner, Rajasthan, India.

[2]Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, Delhi, India.

[3]Division of Food Science & Post-harvest Technology, ICAR-IARI, New Delhi, Delhi, India.

***Author for correspondence:**

aksureja@gmail.com

Received: 22/08/2022 **Revised:** 12/03/2023

Accepted: 13/03/2023

How to cite this article: Ram, H., Sureja, A.K., Dey, S.S., Kar, A. (2023). Breeding for Higher Ascorbic Acid and Mineral Nutrients in Snowball Cauliflower (*Brassica oleracea* var. *botrytis* L.). *Indian J. Plant Genetic Resources*. 36(2), 256-264.

DOI: 10.5958/0976-1926.2023.00036.2.08

Introduction

Cauliflower (*Brassica oleracea* var. *botrytis* L., 2n = 2x = 18) is a popular brassica vegetable grown mostly as winter season crop in India. It is used as a vegetable in curries, soups, pickles (Rakshita *et al*., 2021; Dey *et al*., 2013). Pieces of cauliflower (buttons) can be fried with *besan* for preparation of *pakoras*. Cauliflower is grown worldwide in 1.42 mha area with annual production of 26.50 million tonnes. China (40.5%) and India (33.2%) are the major global producers of cauliflower (FAOSTAT, 2018). In India, cauliflower is grown in 0.47 mha area with annual production of 9.22 million tonnes (Agricultural Statistics at a Glance 2021). Cauliflower is classified into four maturity groups, group I (September to early November), group II (mid-November to early December), group III (mid-December to mid-January) and group IV (mid-January to early March). First three groups are Indian cauliflower and the last group is called Erfurt or alpha types *i.e.,* snowball (late group) type as per Swarup and Chatterjee (1972). In this crop, breeding work has

primarily been focussed on early and mid-groups, but not on the snowball group due to insufficient genetic variability and specific geographical conditions for seed production. Snowball or European cauliflower is the main vegetable crop grown during winter season in the Indian sub-continent.

The macro- and micronutrients are vital for human beings and plants. Knowledge of mineral element concentrations in plants would be beneficial in monitoring and managing the plant, human and animal nutrition balance in a food-chain cycle (Ram *et al*., 2018). Billions of people in developing countries suffer from micronutrient malnutrition or 'hidden hunger' caused by intake of insufficient micronutrients such as Vitamin-A, Zn, and Fe (Harvest Plus, 2007). Vegetable *Brasiccas* are good sources of different phytochemicals and mineral elements. Cauliflower supplies ascorbic acid (47.14 mg/100 g) and minerals like calcium (25.16 mg/100 g), magnesium (23.08 mg/100 g), phosphorus (47.33 mg/100 g), potassium (329 mg/100 g), manganese (0.23 mg/100 g), iron (0.96 mg/100 g), copper (0.05 mg/100 g) and zinc (0.31 mg/100 g) (Longvah *et al.* 2017).

The line × tester analysis is one of the most appropriate approaches in preliminary screening of the materials for gene action effects and variances since it can evaluate more germplasm at a time and also provides information for selecting suitable parents and adopting breeding methodology for improving the crop. Most of the genetic studies and breeding efforts have been targeted to limited vegetable crops such as peppers, tomatoes, eggplant and cucurbits. The information regarding breeding for mineral elements in cauliflower is limited. Therefore, the present investigation has been undertaken to determine the gene action for mineral elements using line × tester mating design in snowball cauliflower to suggest suitable breeding approaches for increasing the mineral content of cauliflower curd.

Materials and Methods

Five genetically diverse Ogura based CMS lines of snowball cauliflower *viz*. Ogu 13-01, Ogu 101, Ogu 103, Ogu 119 and Ogu 13-85 and seven testers *viz*. Kt-18, Kt-22, DB-1305, DB-187, Lalchowk Maghi, Sel-26 and Suprima × Late were crossed in line × tester mating scheme (Kempthorne, 1957) to obtain 35 F_1 hybrid combinations during spring summer season at IARI Regional Station, Katrain, H.P. The 35 F_1 hybrids along with their twelve parental lines were evaluated in a randomized block design with three replications during the winter season at the research farm of Division of Vegetable Science, Indian Agricultural Research Institute, New Delhi. Seedlings (38 days old) were transplanted providing inter- and intra-row spacing of 45 cm between plants. All recommended package of practices were followed to grow a healthy successful crop (Thamburaj and Singh, 2001). Each treatment comprised of 5 rows and 8 plants per row and was replicated thrice. Five curds of each genotype in replicated trial were chopped at fresh marketable stage and homogenized for analysis of ascorbic acid and mineral nutrients.

Ascorbic Acid Content Estimation

Ascorbic acid was determined by titrating a known weight of sample with 2,6-dichlorophenol indophenol dye using meta-phosphoric acid as a stabilizing agent (Albrecht, 1993). 3% metaphosphoric acid (HPO_3) was prepared by dissolving the sticks or pellets of HPO_3 in distilled water and diluting it to 100 mL with 3% HPO_3. Ascorbic acid standard is prepared by weighing 100 mg of L-ascorbic acid, dissolving in 3% HPO_3, and making the final volume 100 mL. This was further diluted by taking 10 mL of the above mixture to 100 mL with 3% HPO_3 (1 mL = 0.1 mg of ascorbic acid). For making dye solution, 50 mg of the sodium salt of 2,6-dichlorophenol indophenol in approximately 150 mL of hot glass distilled water containing 42 mg of sodium bicarbonate was dissolved. It was cooled and diluted with distilled water to 200 mL and stored in a refrigerator.

For standardization of dye, 5 mL of standard ascorbic acid solution was taken and 5 mL of HPO_3 was added into it. A micro burette was filled with the dye. It was then titrated with the dye solution to a pink color which persisted for about 15 seconds. The dye factor was determined *i.e.*, mg of ascorbic acid per mL of the dye, using the following formula:

$$\text{Dye factor} = \frac{0.5}{\text{Titre value}}$$

Five gram of cauliflower curd sample was taken and crushed with some amount of 3% HPO_3 in mortar and pestle. The volume was made up to 100 mL with 3% HPO_3 and then filtered and centrifuged. The prepared sample was taken in a conical flask and titrated against standardized dye. The titre value was noted and used for calculation of ascorbic acid content in the sample.

The ascorbic acid content of the sample was calculated with the following formula:

$$\text{Ascorbic acid content (mg/100g)} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Vol. made up}}{\text{Aliquot of extract} \times \text{volume of sample taken}} \times 100$$

Mineral Nutrients Analyses

Procedure for sample preparation, digestion and estimation of nutrients as described by Tandon (1998) and Jones (2001) were followed. Composite samples of five curds selected randomly from each replication genotype were collected at the edible maturity stage. The curds were chopped into slices, homogenized and 100g of fresh material was kept in oven at 60°C till the samples were dried properly. Dried pieces were then grinded thoroughly in mortar and pestle to a fine powder, passed through a 1-mm sieve, and stored in a butter paper bag inside the desiccator until further use.

Just before the use, the powder was again heated for 2 to 3 hours at 60°C to make it free from any moisture and

then 2.0 g dry powder was transferred into the digestion tube. Tissue samples were pre-digested by adding 20 mL of concentrated HNO_3 in digestion tubes. Samples were then heated at 60°C for 30 minutes, 120°C for 30 minutes and 255°C for 2 hours or till the digestion mixture became transparent on the digestion block. Tubes were then removed from the block and allowed to cool down to room temperature. Volume was made up to 20 mL by adding concentrated HNO_3. The volume of 1-mL taken from digested solution was diluted to 100 mL by adding double distilled water and then it was filtered through Whatman filter paper (number 42). Now samples were used for the estimation of Ca, Mg, Na, K, S, Fe, Mn and Zn. Standard solutions for each nutrient element were also prepared to make standard curves for the respective element.

Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (K), Iron (Fe), Manganese (Mn) and Zinc (Zn) were determined using atomic absorption spectrophotometer (AAS). Absorptions were recorded at 422.7, 285.2, 589.0, 766.5, 248.3, 279.5 and 213.9 nm for Ca, Mg, Na, K, Fe, Mn and Zn, respectively using standard specifications and their respective standard curves. All the elemental concentrations were expressed as per 100 g fresh weight (FW) basis.

In the estimation of total sulphur in the plant sample, wet digest was taken from di-acid digestion method and then determined by barium sulfate turbidimetry method. During wet digestion of the sample, all the plant sulfur is converted to sulfate form, which when treated with $BaCl_2$ is precipitated as $BaSO_4$. This provides turbidity to the solution which is proportional to the amount of sulphate present. Measurement of this turbidity provides the means for the quantitative determination of sulphur. Gum acacia solution was added to help stabilize turbidity.

Statistical analysis for estimation of analysis of variance, components of variance (coefficient of variance, genotypic and phenotypic coefficient of variation), and components of genetic variance (additive variance, σ^2A and dominance variance, σ^2D) was carried out as per Kempthorne (1957) and Singh and Chaudhary (1995).

Results and Discussion

Evaluation of Parents for Ascorbic Acid and Different Mineral Nutrients

The mean value of CMS lines for ascorbic acid (mg/100 g) content ranged from 46.67 (Ogu 103) to 81.26 (Ogu 119). The Na, K, S, Ca and Mg content (mg/100g) ranged from 14.02 (Ogu 101) to 24.37 (Ogu 13-85), 77.22 (Ogu 101) to 182.32 (Ogu 103), 11.12 (Ogu 119) to 29.48 (Ogu 103), 8.84 (Ogu 101) to 23.19 (Ogu 103) and 7.45 (Ogu 101) to 13.22 (Ogu 103), respectively. The Fe, Zn and Mn content (µg/100 g) ranged from 172.20 (Ogu 101) to 645.03 (Ogu 13-01), 45.30 (Ogu 119) to 313.33 (Ogu 103) and 49.43 (Ogu 101) to 235.00 (Ogu 103), respectively. The mean value of testers for ascorbic acid (mg/100 g) content varied from 37.28 (Sel-26) to 70.84 (Kt-22). The Na, K, S, Ca and Mg content (mg/100 g) ranged from 36.81 (Lalchowk Maghi) to 98.03 (Kt-18), 142.57 (Lalchowk Maghi) to 277.52 to (Kt-18), 16.32 (Lalchowk Maghi) to 42.87 (Kt-18), 23.22 (DB-1305) to 43.73 (Kt-18) and 14.23 (Lalchowk Maghi) to 29.44 (Kt-18), respectively. The micronutrients Fe, Zn and Mn content (µg/ 100 g) ranged from 544.33 (Lalchowk Maghi) to 1424.93 (Sel-26), 108.87 (Lalchowk Maghi) to 458.70 (Sel-26) and 147.73 (DB-1305) to 343.50 (Kt-22), respectively (Table 1). Among the CMS lines, Ogu 103 had highest content of K, S, Ca, Mg, Zn and Mn. Ogu 101 recorded minimum values for Na, K, Ca, Mg, Fe and Mn contents. CMS line Ogu 13-01 was second best for Na (21.28 mg/100 g FW), K (137.81 mg/100 g FW), S (21.15 mg/100 g FW) and Ca (15.97 mg/100 g FW). Among testers, Kt-18 had highest Na, K, S, Ca and Mg, Sel-26 recorded highest Fe and Zn, while Kt-22 had the maximum ascorbic acid and Mn content. Kt-18 also had second highest value for Fe (1413.43 µg/100 g) and Zn (292.40 µg/100 g) contents. The best-performing lines and testers can be utilized as parents to develop mineral nutrient-rich hybrids.

Evaluation of Hybrids for Ascorbic Acid and Different Mineral Nutrients

The *per se* performance of 35 F_1 hybrids for ascorbic acid and different mineral nutrients is presented in Table 2. The ascorbic acid content (mg/100 g FW) in hybrids ranged from 31.79 (Ogu 103 × Sel-26) to 94.05 (Ogu 101 × Sel-26). The hybrid Ogu 101 × Sel-26 has a maximum concentration (mg/100 g) of ascorbic acid (94.05), followed by Ogu 119 × Lalchowk Maghi (87.58), Ogu 101 × DB-1305 (82.29) and Ogu 101 × Suprimax Late (81.07). The hybrid Ogu 101 × Sel-26 also recorded higher concentration of ascorbic acid in comparison of the best parent i.e., Ogu119 (81.26) and checks PSB Kt 25 (62.21) and Mamta (52.81).

The mean value for Na, K, S, Ca and Mg (mg/100g FW) ranged from 13.92 (Ogu 13-01 × Suprimax Late) to 98.63 (Ogu 103 × Sel-26), 93.46 (Ogu 101 × DB-1305) to 300.18 (Ogu 13-01 × Sel-26), 18.21 (Ogu 101 × DB-187) to 49.34 (Ogu 101 × Lalchowk Maghi), 12.63 (Ogu 103 × Suprimax Late) to 54.65 (Ogu 119 × Suprimax Late), 7.55 (Ogu 13-85 × Kt-18) to 35.60 (Ogu 13-85 × DB-187), respectively.

The Na content (mg/100 g FW) was recorded highest in the hybrid Ogu 103 × Sel-26 (98.63), followed by Ogu 101 × DB-1305 (97.49), Ogu 119 × Suprimax Late (94.95) and Ogu 13-85 × DB-1305 (92.72). Potassium content (mg/100 g FW) was maximum in the hybrid Ogu 13-01 × Sel-26 (300.18), followed by Ogu 101 × Lalchowk Maghi (299.49), Ogu 13-01 × Kt-18 (290.13), Ogu 119 × Suprimax Late (283.56) and Ogu 13-01 × DB-187 (282.47). For S content (mg/100 g FW), the highest value was recorded in the hybrid Ogu 101 × Lalchowk Maghi (49.34), followed by Ogu 103 × DB-187

Table 1: Mean performance of parental lines for ascorbic acid and different mineral nutrients in snowball cauliflower

Nutrients *Genotypes*	*Ascorbic acid* *(mg/100 g FW)*	*Na* *(mg/100 g FW)*	*K* *(mg/100 g FW)*	*S* *(mg/100 g FW)*	*Ca* *(mg/100 g FW)*	*Mg* *(mg/100 g FW)*	*Fe* *(µg/100 g FW)*	*Zn* *(µg/100 g FW)*	*Mn* *(µg/100 g FW)*
Lines									
Ogu 13-01	47.64^{c}	21.18^{b}	137.81^{b}	21.15^{b}	15.97^{b}	12.06^{b}	645.03^{a}	194.30^{b}	129.53^{c}
Ogu 101	66.67^{b}	14.02^{d}	77.22^{e}	12.89^{c}	8.84^{d}	7.45^{e}	172.20^{c}	98.87^{c}	49.43^{d}
Ogu 103	46.67^{c}	19.35^{c}	182.32^{a}	29.48^{a}	23.19^{a}	13.22^{a}	600.03^{a}	313.33^{a}	235.00^{a}
Ogu 119	81.26^{a}	18.95^{c}	85.26^{b}	11.12^{d}	12.79^{c}	9.49^{d}	331.57^{b}	45.30^{d}	181.20^{b}
Ogu 13-85	62.39^{b}	24.37^{a}	127.34^{c}	20.08^{b}	11.96^{c}	10.45^{c}	194.60^{c}	194.60^{b}	129.73^{c}
Mean	60.93	19.57	121.99	18.94	14.55	10.53	388.69	169.28	144.98
Range	46.67-81.26	14.02-24.37	77.22-182.32	11.12-29.48	8.84-23.19	7.45-13.22	172.20-645.03	45.30-313.33	49.43-235.00
SE	2.21	0.29	2.46	0.37	0.28	0.18	15.03	5.08	4.25
CD 5%	4.38	0.57	4.87	0.74	0.55	0.35	29.76	10.06	8.42
CD 1%	5.41	0.71	6.03	0.91	0.68	0.43	36.82	12.45	10.41
Testers									
Kt-18	39.72^{e}	98.03^{a}	277.52^{a}	42.87^{a}	43.73^{a}	29.44^{a}	1413.43^{a}	365.50^{b}	292.40^{b}
Kt-22	70.84^{a}	49.59^{c}	153.25^{f}	32.85^{c}	37.16^{b}	22.47^{c}	1053.57^{b}	274.80^{c}	343.50^{a}
DB-1305	53.68^{b}	88.58^{a}	176.30^{d}	38.82^{b}	23.22^{e}	20.25^{d}	588.93^{c}	147.73^{e}	147.73^{e}
DB-187	41.91^{d}	37.94^{c}	167.88^{e}	23.02^{e}	28.28^{d}	19.04^{e}	654.43^{c}	226.80^{d}	226.80^{c}
Lalchowk Maghi	38.86^{e}	36.81^{c}	142.57^{g}	16.32^{f}	23.96^{e}	14.23^{f}	544.33^{c}	108.87^{f}	163.30^{e}
Sel-26	37.28^{f}	70.42^{b}	250.16^{b}	23.45^{e}	37.26^{b}	25.31^{b}	1424.93^{a}	458.70^{a}	206.80^{d}
Suprimax Late	45.22^{c}	62.21^{b}	203.99^{c}	27.57^{d}	35.05^{c}	20.68^{d}	1347.80^{a}	134.67^{ef}	336.67^{a}
Mean	46.79	63.37	195.95	29.27	32.67	21.63	1003.92	245.30	245.31
Range	37.28-70.84	36.81-98.03	142.57-277.52	16.32-42.87	23.22-43.73	14.23-29.44	544.33-1424.93	108.87-458.70	147.73-343.50
SE	0.420	3.97	2.59	0.484	0.367	0.242	43.63	11.14	5.85
CD 5%	0.83	7.86	5.13	0.96	0.73	0.48	86.39	22.06	11.58
CD 1%	1.03	9.73	6.35	1.19	0.90	0.59	106.89	27.29	14.33
PSB Kt 25 (C)	62.21	35.37	196.66	55.67	19.56	12.34	1078.97	258.73	141.27
Mamta (C)	52.81	36.90	167.44	24.07	34.45	22.59	1164.33	320.80	152.60

CD: Critical difference, SE: Standard error, Values within columns with different letters (superscript) are significantly different according to Duncan's test at P = 0.05.

Table 2: Mean performance of hybrids (crosses) for ascorbic acid and different mineral nutrients in snowball cauliflower

Nutrients *Hybrids*	*Ascorbic acid (mg/100 g FW)*	*Na (mg/100 g FW)*	*K (mg/100 g FW)*	*S (mg/100 g FW)*	*Ca (mg/100 g FW)*	*Mg (mg/100 g FW)*	*Fe (µg/100 g FW)*	*Zn (µg/100 g FW)*	*Mn (µg/100 g FW)*
Ogu 13-01 × Kt-18	51.53^{hijkl}	21.59^{k}	290.13^{ab}	37.05^{efg}	32.99^{de}	7.75^{n}	1248.23^{de}	288.00^{l}	216.00^{cd}
Ogu 13-01 × Kt-22	61.58^{efg}	23.55^{k}	207.79^{cdefgh}	22.28^{qr}	17.94^{hijk}	10.45^{lmn}	509.17^{lmno}	203.87^{no}	101.93^{ghijk}
Ogu 13-01 × DB-1305	67.45^{def}	65.49^{de}	118.89^{ijk}	26.78^{mnopq}	18.77^{hij}	20.66^{gh}	435.40^{nop}	327.00^{j}	130.80^{fghij}
Ogu 13-01 × DB-187	49.77^{ijkl}	43.09^{hij}	282.47^{abc}	35.56^{efghij}	39.17^{c}	28.74^{c}	1775.60^{a}	574.93^{c}	321.40^{ab}
Ogu 13-01 × Lalchowk Maghi	59.02^{fgh}	48.08^{gh}	212.52^{bcdefgh}	37.16^{efg}	33.68^{de}	22.71^{fg}	1256.87^{de}	337.00^{j}	202.20^{de}
Ogu 13-01 × Sel-26	72.65^{cd}	75.35^{bc}	300.18^{a}	33.35^{fghijk}	25.49^{f}	27.26^{cd}	824.59^{fgh}	150.53^{r}	350.57^{a}
Ogu 13-01 × Suprimax Late	62.90^{efg}	13.92^{k}	133.19^{hijk}	26.72^{mnopq}	21.43^{ghi}	13.40^{jkl}	590.47^{jklmn}	204.60^{no}	136.40^{efghi}
Ogu 101 × Kt-18	56.71^{ghijk}	35.30^{ij}	139.56^{hijk}	38.34^{ef}	19.31^{ghij}	12.10^{klm}	767.57^{ghij}	230.50^{m}	127.37^{fghijk}
Ogu 101 × Kt-22	48.84^{klm}	61.21^{ef}	140.23^{hijk}	30.34^{jklmn}	22.89^{fgh}	24.43^{def}	359.83^{opq}	360.47^{hi}	381.60^{a}
Ogu 101 × DB-1305	82.29^{b}	97.49^{a}	93.46^{k}	44.02^{bcd}	33.29^{de}	26.78^{cd}	699.20^{hijk}	336.43^{j}	233.50^{cd}
Ogu 101 × DB-187	46.86^{lmno}	14.32^{k}	109.17^{ijk}	18.21^{r}	18.67^{hij}	13.26^{jkl}	476.57^{lmnop}	352.97^{i}	144.73^{efg}
Ogu 101 × Lalchowk Maghi	39.89^{nopq}	54.80^{fg}	299.49^{a}	49.34^{a}	33.49^{de}	24.41^{def}	1384.53^{cd}	642.10^{a}	204.73^{de}
Ogu 101 × Sel-26	94.05^{a}	66.59^{cde}	252.67^{abcdef}	35.94^{efghi}	33.79^{de}	26.37^{cde}	656.13^{hijkl}	310.93^{k}	280.93^{bc}
Ogu 101 × Suprimax Late	81.07^{b}	18.17^{k}	165.05^{ghijk}	38.67^{ef}	19.84^{ghij}	14.90^{ijk}	749.90^{ghij}	183.07^{pq}	137.07^{efghi}
Ogu 103 × Kt-18	38.51^{opq}	65.11^{de}	179.80^{efghij}	31.14^{hijklmn}	13.01^{kl}	8.08^{n}	632.71^{ijklm}	189.60^{opq}	71.13^{hijk}
Ogu 103 × Kt-22	46.12^{lmnop}	63.58^{ef}	109.36^{ijk}	22.97^{pqr}	36.80^{cd}	14.06^{jk}	170.30^{r}	132.60^{s}	56.77^{k}
Ogu 103 × DB-1305	70.02^{de}	36.71^{ij}	201.59^{defgh}	38.08^{ef}	25.66^{f}	26.10^{cde}	1174.40^{e}	276.93^{l}	64.33^{ijk}
Ogu 103 × DB-187	48.04^{klmn}	17.27^{k}	215.22^{bcdefgh}	48.03^{ab}	22.51^{fgh}	9.35^{mn}	557.43^{klmn}	198.47^{op}	104.77^{ghijk}
Ogu 103 × Lalchowk Maghi	40.22^{mnopq}	23.65^{k}	136.26^{hijk}	25.84^{nopq}	26.69^{f}	16.64^{ij}	950.27^{f}	383.87^{g}	54.93^{k}
Ogu 103 × Sel-26	31.79^{q}	98.63^{a}	117.50^{ijk}	31.77^{ghijklm}	46.00^{b}	10.41^{lmn}	1501.47^{bc}	605.67^{b}	72.83^{ghijk}
Ogu 103 × Suprimax Late	37.94^{pq}	73.95^{cd}	167.61^{ghijk}	37.92^{ef}	12.63^{l}	24.56^{def}	782.80^{fghi}	182.93^{pq}	122.80^{fghijk}
Ogu 119 × Kt-18	72.11^{d}	44.60^{hi}	167.69^{ghijk}	24.62^{opq}	22.18^{fgh}	22.04^{fg}	921.00^{fg}	179.60^{q}	60.20^{jk}
Ogu 119 × Kt-22	57.68^{ghij}	69.16^{cde}	280.05^{abcd}	41.16^{cde}	15.30^{jkl}	15.65^{ij}	210.70^{qr}	232.87^{m}	70.23^{hijk}
Ogu 119 × DB-1305	51.65^{hijkl}	37.37^{ij}	175.44^{fghijk}	31.25^{hijklmn}	25.09^{f}	16.64^{ij}	457.47^{mnop}	189.80^{opq}	57.40^{k}
Ogu 119 × DB-187	59.30^{fgh}	42.07^{hij}	151.63^{hijk}	27.73^{lmnopq}	47.06^{b}	14.24^{jk}	348.87^{opq}	217.40^{mn}	131.47^{fghij}
Ogu 119 × Lalchowk Maghi	87.58^{ab}	40.95^{hij}	145.86^{hijk}	27.93^{klmnop}	16.33^{ijkl}	23.17^{efg}	639.23^{ijklm}	189.80^{opq}	189.80^{def}
Ogu 119 × Sel-26	62.16^{efg}	54.68^{fg}	184.22^{efghi}	45.67^{abc}	24.43^{fg}	20.18^{gh}	1147.87^{e}	180.47^{q}	121.53^{fghijk}
Ogu 119 × Suprimax Late	42.99^{lmnop}	94.95^{a}	283.56^{abc}	36.55^{efgh}	54.65^{a}	32.58^{b}	1581.43^{b}	374.70^{gh}	355.47^{a}
Ogu 13-85 × Kt-18	58.17^{ghi}	19.33^{k}	98.69^{jk}	22.81^{pqr}	45.93^{b}	7.55^{n}	1110.63^{e}	423.73^{f}	282.13^{bc}

Ogu 13-85 × Kt-22	65.44^{defg}	69.33^{cde}	233.62^{abcdefg}	27.49^{mnopq}	16.75^{jkl}	17.80^{hi}	881.80^{fg}	618.67^{b}	216.93^{cd}
Ogu 13-85 × DB-1305	72.92^{cd}	92.72^{a}	276.21^{abcd}	29.35^{klmno}	31.51^{e}	19.90^{gh}	474.63^{lmnop}	136.20^{rs}	136.20^{efghi}
Ogu 13-85 × DB-187	59.35^{fgh}	34.32^{l}	140.63^{hijk}	39.96^{de}	26.33^{f}	35.60^{a}	1448.50^{bc}	507.60^{d}	361.93^{a}
Ogu 13-85 × Lalchowk Maghi	49.22^{jkl}	62.40^{ef}	168.39^{ghijk}	30.52^{ijklmn}	40.43^{c}	9.93^{mn}	313.97^{pqr}	489.67^{e}	254.80^{cd}
Ogu 13-85 × Sel-26	44.37^{lmnop}	83.54^{b}	150.71^{hijk}	33.18^{fghijkl}	31.67^{de}	15.10^{ijk}	1245.40^{de}	330.30^{l}	141.67^{efgh}
Ogu 13-85 × Suprimax Late	80.54^{bc}	36.00^{jl}	258.17^{abcde}	27.43^{mnopq}	18.81^{hij}	9.21^{mn}	1433.70^{bc}	151.07^{r}	65.50^{ijk}
Mean	58.59	51.41	188.20	33.00	27.73	18.34	849.10	305.55	170.34
Range	31.79-94.05	13.92-98.63	93.46-300.18	18.21-49.34	12.63-54.65	7.55-35.60	170.3-1775.60	132.6-642.10	54.93-381.60
SE	2.69	3.06	23.66	1.67	1.60	1.04	55.43	5.42	21.28
CD 5%	5.33	6.06	46.85	3.31	3.17	2.06	109.75	10.73	42.13
CD 1%	6.59	7.50	57.97	4.09	3.92	2.55	135.80	13.28	52.14
PSB K 25 (C)	62.21	35.37	196.66	55.67	19.56	12.34	1078.97	258.73	141.27
Mamta (C)	52.81	36.90	167.44	24.07	34.45	22.59	1164.33	320.80	152.60

CD: Critical difference, SE: Standard error, Values within columns with different letters (superscript) are significantly different according to Duncan's test at P = 0.05.

(48.03), Ogu 119 × Sel-26 (45.67), Ogu 101 × DB-1305 (44.02) and Ogu 119 × Kt-22 (41.16). Calcium content (mg/ 100 g FW) was highest in Ogu 119 × Suprimax Late (54.65), followed by Ogu 119 × DB-187 (47.06), Ogu 103 × Sel-26 (46.00) and Ogu 13-85 × Kt-18 (45.93). The highest value for Mg content (mg/100 g FW) was exhibited by the hybrid Ogu 13-85 × DB-187 (35.60), followed by Ogu 119 × Suprimax Late (32.58), Ogu 13-01 × DB-187 (28.74) and Ogu 13-01 × Sel-26 (27.26) (Table 2). The hybrids Ogu 103 × Sel-26 (98.63), Ogu 13-01 × Sel-26 (300.18), Ogu 101 × Lalchowk Maghi (49.34), Ogu 119 × Suprimax Late (54.65) and Ogu 13-85 × DB-187 (35.60) recorded higher content of Na, K, S, Ca and Mg, respectively (mg/100 g FW) over the best parent Kt-18 (Na: 98.03, K: 277.52, S: 42.87, Ca: 43.73 and Mg: 29.44) and checks: PSB Kt 25 (Na: 35.37, K: 196.66, S: 55.67, Ca: 19.56 and Mg: 12.34) and Mamta (Na: 36.90, K: 167.44, S: 24.07, Ca: 34.45 and Mg: 22.59).

For Fe, Zn and Mn content (µg/100 g FW), the mean value ranged from 170.30 (Ogu 103 × Kt-22) to 1775.60 (Ogu 13-01 × DB-187), 132.60 (Ogu 103 × Kt-22) to 642.10 (Ogu 101 × Lalchowk Maghi) and 54.93 (Ogu 103 × Lalchowk Maghi) to 381.60 (Ogu 101 × Kt-22), respectively (Table 2). The maximum value for Fe content (µg/100g FW) was recorded in the hybrid Ogu 13-01 × DB-187 (1775.60), followed by Ogu 119 × Suprimax Late (1581.43), Ogu 103 × Sel-26 (1501.47), Ogu 13-85 x DB-187 (1448.50), Ogu 13-85 × Suprimax Late (1433.70), Ogu 101 × Lalchowk Maghi (1384.53), Ogu 13-01 × Lalchowk Maghi (1256.87) and Ogu 13-01 × Kt-18 (1248.23). Zinc content (µg/100 g FW) was highest in the hybrid Ogu 101 × Lalchowk Maghi (642.10), followed by Ogu 13-85 × Kt-22 (618.67), Ogu 103 × Sel-26 (605.67) and Ogu 13-01 × DB-187 (574.93). The maximum value for Mn content (µg/100g FW) was recorded in the hybrid Ogu 101 × Kt-22 (381.60), followed by Ogu 13-85 × DB-187 (361.93), Ogu 119 × Suprimax Late (355.47), Ogu 13-01 × Sel-26 (350.57) and Ogu 13-01 × DB-187 (321.40). The hybrids Ogu 13-01 × DB-187 (1775.60) and Ogu 101 × Lalchowk Maghi (642.10) exhibited higher Fe and zn content, respectively (µg/100 g FW) in comparison of the best parent: Sel-26 (1424.93) and checks: PSB Kt 25 (Fe: 1078.97 and Zn: 258.73) and Mamta (Fe: 1164.33 and Zn: 320.80). The hybrid Ogu 101 × Kt-22 (381.60) has higher concentration of Mn over the best parent (Kt-22) and checks: PSB Kt 25 (141.27) and Mamta (152.60).

The hybrids Ogu 101 × Sel-26, Ogu 103 × Sel-26, Ogu 13-01 × Sel-26, Ogu 101 × Lalchowk Maghi, Ogu 119 × Suprimax Late, Ogu 13-85 × DB-187, Ogu 13-01 × DB-187, Ogu 101 × Lalchowk Maghi and Ogu 101 × Kt-22 have the highest *per se* performance for ascorbic acid, Na, K, S, Ca, Mg, Fe, Zn and Mn concentration, respectively. Besides highest content of Fe, the hybrid Ogu 13-01 × DB-187 also showed high concentrations of Mg, K, Zn and Mn. Hybrid Ogu 101 × Lalchowk Maghi recorded maximum S and Zn content and had high K and Fe content. The hybrid Ogu 103 × Sel-26 exhibited the highest Na content and had high Ca, Fe and

Table 3: Genetic analysis for ascorbic acid and different mineral nutrients in snowball cauliflower

Source of variation	*Ascorbic Acid (mg/100 g FW)*	*Na (mg/100 g FW)*	*K (mg/100 g FW)*	*S (mg/100 g FW)*	*Ca (mg/100 g FW)*	*Mg (mg/100 g FW)*	*Fe (µg/100 g FW)*	*Zn (µg/100 g FW)*	*Mn (µg/100 g FW)*
Mean sum of squares									
Parents (P)	618.22**	2476.80**	10563.51**	288.66**	389.86**	141.52**	620022.55**	43847.47**	23633.19**
Crosses (C)	703.99**	1919.21**	12435.60**	171.98**	335.60**	169.28**	552179.09**	65465.56**	29977.39**
Parents v/s. Crosses	937.70**	1059.22**	14261.16**	1731.87**	182.78**	47.86**	276368.72**	226563.90**	29486.32**
Males (T)	621.35**	42.67*	5454.41**	161.16**	89.31**	15.13**	148634.90**	31760.67**	14261.79**
Female (L)	427.65**	1715.05**	7752.74**	266.30**	176.71**	69.80**	485623.37**	50786.55**	19138.67**
Male v/s Female (L×T)	1749.19**	16783.87**	47864.56**	932.82**	2870.99**	1077.46**	3311968.30**	50560.18**	88085.85**
Components of variance									
Mean	57.08	49.80	182.31	30.95	27.06	18.00	823.18	282.08	178.81
Range	31.79	13.92	77.22	11.12	8.84	7.45	170.30	45.30	49.43
	94.05	98.63	300.18	49.34	54.65	35.60	1775.60	642.10	381.60
CV %	5.74	7.61	13.79	6.24	6.97	6.75	7.91	20.66	12.87
σ^2g	688.56	2033.85	12027.61	233.79	345.25	160.01	562406.65	63798.15	28449.62
σ^2P	699.29	2048.23	12659.46	237.52	348.81	161.48	566645.93	67195.54	28979.52
h^2_{bs}	0.98	0.99	0.95	0.98	0.99	0.99	0.99	0.95	0.98
G. C. V.%	26.33	52.10	33.81	28.29	39.44	40.38	52.40	50.30	53.95
P. C. V.%	26.95	52.65	36.51	28.97	40.05	40.94	52.99	54.38	55.47
G. C. V. / P. C. V.	0.98	0.99	0.93	0.98	0.98	0.99	0.99	0.92	0.97
Components of genetic variance									
σ^2_{gca}	12.04	37.79	-464.06	-5.36	-15.63	-1.94	-74.34	459.80	945.80
σ^2_{sca}	218.18	538.27	4775.42	65.77	137.06	58.62	177555.83	19623.61	8711.24
$\sigma^2_{gca}/\sigma^2_{sca}$	0.06	0.07	-0.10	-0.08	-0.11	-0.03	-0.0004	0.02	0.11
σ^2A	24.072	75.587	-928.121	-10.713	-31.261	-3.875	-148.68	919.608	1,891.60
σ^2D	218.178	538.27	4775.42	65.767	137.061	58.617	177555.83	19623.61	8711.24

**Significant at p = 0.01
*Significant at p = 0.05

Zn concentrations. Besides highest content of Mg, the hybrid Ogu 13-85 × DB-187 also recorded high concentrations of S, Fe, Mn and Zn.

Analysis of Variance

The partitioning of mean squares into replications, lines, testers and line × tester interactions revealed that the mean sum of squares due to lines (female parents), testers (male parents) and lines × testers interactions were significant for all of the parameters under study. The parents v/s crosses (heterosis) mean square was highly significant for all the traits which indicated the expression of heterotic effects in the developed hybrids. The analysis of variance showed significant differences among the treatments for vitamin C and all mineral nutrients under study. Further, the parents and parents versus crosses also differed significantly for vitamin C and these mineral nutrients. The mean sum of squares due to lines and testers and lines versus testers interactions was highly significant for the estimated vitamin C and all mineral nutrients. Highly significant differences were also observed among the crosses (line × tester) for the vitamin C and mineral nutrients under study. Highly significant mean squares for lines indicate the existence of additive variance. The line × tester interaction showed highly significant mean squares for vitamin C and all the mineral elements under study, indicating the prevalence of non-additive variance. Similar results were obtained while analyzing different mineral elements by Singh *et al.* (2012) in cabbage and by Ram *et al.* (2017) in snowball cauliflower.

Components of Variance

The mean, range, coefficient of variance (C.V%), genotypic (σ^2g) and phenotypic variance (σ^2p), heritability in broad sense (h^2_{bs}), genotypic and phenotypic coefficient of variance (G.C.V & P.C.V%) and the ratio of G.C.V./ P.C.V. are presented in Table 3. The variance widely varied from one trait to another since the coefficient of variation (C.V%) ranged from 5.74 to 20.66%. The variance for a particular trait depends upon the genotype × environment interactions. The highest C.V. (20.66%) was recorded for zinc, followed by potassium (K) and manganese (Mn), suggesting that these three minerals had the highest variation among the studied genotypes. These parameters are obvious to exhibit higher variance due to their highly quantitative nature. On the contrary, the lowest variation (5.74%) was observed for ascorbic acid (vitamin C).

The difference between the genotypic (σ^2g) and phenotypic (σ^2p) variances indicated the contribution of environmental variance effects. Low values of differences between σ^2p and σ^2g indicated the lesser environmental effect on the character. Selection based on the phenotypic values will be effective only when the phenotypic and genotypic values are nearly identical. In this respect, all the characters studied have closer values of σ^2g and σ^2p as well as G.C.V.% and P.C.V.%, which is confirmed by the estimated G.C.V/P.C. V. ratios ranging from 0.92 to 0.99, and broad sense heritability (h^2_{bs}) ranging from 0.95 to 0.99 suggesting less effect of environment. Arivalagan *et al.* (2013) also observed high broad sense heritability (84.44–97.07%) for potassium, magnesium, copper, iron and zinc in eggplant.

Components of Genetic Variance (gene action)

Using line × tester mating design, the genetic variance could be divided into components of genetic variance *i.e.,* additive and non-additive genetic variances. Both the lines variance (σ^2L) and tester variance (σ^2T) determine the σ^2gca which is an indicator of additive and additive × additive element of the genetic variance. While, the line x tester variance ($\sigma^2L \times T$) determines the σ^2sca and it indicates the non-additive genetic elements, including dominance. According to Kalloo (1988), the additive (σ^2A) and dominance (σ^2D) were the most important elements.

The estimates of variance components for earliness and yield attributing traits are presented in Table 1. The values of σ^2gca were lower than the σ^2sca for all the parameters. For potassium (K), sulphur (S), calcium (Ca), magnesium (Mg) and iron (Fe) the σ^2gca was in negative direction. The proportions of σ^2gca/σ^2sca were less than unity (<1) in all parameters, indicating the preponderance of non-additive variance in the inheritance of these traits, suggesting that heterosis breeding is effective for improving these traits. Similarly, σ^2D was greater than σ^2A for all the parameters.

The results of present findings are in agreement with the earlier work of Verma and Singh (2019) and Kumari (2014) in cauliflower. Singh *et al.* (2012) also found the role of non-additive gene actions in the accumulation of Fe, Zn, Mn, K and Ca in cabbage head. Sit and Sirohi (2000) reported overdominance for phosphorus, calcium, iron and vitamin C in bottle gourd and found more frequency of dominant alleles for these traits. Saha *et al*. (2018) studied gene action of nutritional and quality traits (acidity, vitamin C, TSS, sodium, potassium, zinc, manganese, copper, iron, magnesium, calcium, respiration rate and ethylene emission rate) in muskmelon and reported the predominance of dominant gene action over additive gene action for all these parameters. The value of σ^2gca was negative for K, S, Ca, Mg and Fe, probably because of the high degree of mean square value for line × tester interaction. The results indicated the importance of heterosis breeding and recurrent selection schemes for effectively utilizing non-additive genetic variance to improve such traits.

Summary and Conclusion

Among the CMS lines, Ogu 103 had highest content of K, S, Ca, Mg, Zn and Mn. Ogu 101 recorded minimum values for Na, K, Ca, Mg, Fe and Mn contents. The hybrids Ogu 101 × Sel-26, Ogu 103 × Sel-26, Ogu 13-01 × Sel-26, Ogu 101 × Lalchowk Maghi, Ogu 119 × Suprimax Late, Ogu 13-85 × DB-187, Ogu 13-01 × DB-187, Ogu 101 × Lalchowk Maghi

and Ogu 101 × Kt-22 have the highest *per se* performance for ascorbic acid, Na, K, S, Ca, Mg, Fe, Zn and Mn concentration, respectively. The present investigation revealed the predominance of dominance component of variance for vitamin C and mineral nutrients in snowball cauliflower curd. The general and specific combining abilities variance was highly significant, indicating the importance of both additive as well as non-additive gene actions. However, the ratios of σ^2gca/ σ^2sca revealed the preponderance of non-additive variance for the inheritance of traits studied. The results of the experiment suggest the possibility of improvement of these traits through recurrent selection and hybridization.

Acknowledgments

The first author is thankful to the Indian Council of Agricultural Research (ICAR) and ICAR-IARI, New Delhi, India for the grant of Junior Research Fellowship and facilities for conducting the study, respectively. The authors thank Head, IARI Regional Station, Katrain, Himachal Pradesh for providing CMS lines and male fertile lines used in the study and facility for development of the hybrids.

References

Agricultural Statistics at a Glance 2021 (2022). Ministry of Agriculture & Farmers Welfare, Department of Agriculture & Farmers Welfare, Directorate of Economics & Statistics, Government of India. pp. 159

Albrecht, J. A. (1993). Ascorbic acid retention in lettuce. *J. Food Qual.* **16:** 311–316.

Arivalagan, M., Bhardwaj, R., Gangopadhyay, K. K., Prasad, T.V., & Sarkar, S. K. (2013). Mineral composition and their genetic variability analysis in eggplant (*Solanum melongena* L.) germplasm. *J. Appl. Bot. Food Qual.* **86:** 99-103.

Dey, S. S., Bhatia, R., Sharma, S. R., & Sureja, A. K. (2013). Effects of chloroplast substituted Ogura male sterile cytoplasm on the performance of cauliflower (*Brassica oleracea* var. *botrytis* L.) F hybrids. *Sci. Hortic.* **157:** 45–51.

FAOSTÀT (2018). Food and Agriculture Organization of United Nations. Italy, Rome.

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* **9:** 463-493.

Harvest Plus (2007). http://www.harvestplus.org/micronut.html

Hayman, B. I. (1954). The theory and analysis of diallel crosses. *Biom. J.* **10:** 235–244.

Jones, J. B. (2001). *Plant analysis handbook*: *A practical sampling, preparation, analysis and interpretation guide*. Micro-macro publishing Inc., Athens, Georgia, USA.

Kalloo (1988). *Vegetable breeding,* Vol. 1. CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida, 33431, pp 61.

Kempthorne, O. (1957). *An introduction to genetic statistics*. John Wiley & Sons, Inc., New York, pp. 468–473.

Kumari, R. (2014). Heterosis and combining ability studies for curd yield and component traits in cauliflower (*Brassica oleracea* var. *botrytis* L.). M.Sc. thesis, Department of Vegetable Science and Floriculture, CSKHPKV, Palampur, pp. 90.

Longvah, T., Ananthan, R., Bhaskarachary, K., & Venkaiah, K. (2017). *Indian food composition tables*. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India.

Rakshita, K. N., Singh, S., Verma, V. K., Sharma, B. B., Saini, N., Iquebal, M. A., & Behera, T. K. (2021). Understanding population structure and detection of QTLs for curding-related traits in Indian cauliflower by genotyping by sequencing analysis. *Funct. Integr. Genomics,* **21:** 679–693.

Ram, H., Dey, S. S., Gopala Krishnan, S., Kar, A., Bhardwaj, R., Arun Kumar, M. B., Kalia, P., & Sureja, A. K. (2017). Heterosis and combining ability for mineral nutrients in snowball cauliflower (*Brassica oleracea* var. *botrytis* L.) using Ogura cytoplasmic male sterile lines. *Proceedings of National Academy of Sciences, India-Section B: Biol.* **88(4):** 1367–1376.

Saha, K., Choudhary, H., Mishra, S., & Mahapatra, S. (2018). Gene action of nutritional and quality traits in muskmelon (*Cucumis melo* L.). *Int. J. Chem. Stud.* **6(3):** 3094-3097.

Singh, B. K., Sharma, S. R., & Singh, B. (2012). Genetic combining ability for concentration of mineral elements in cabbage head (*Brassica oleracea* var. *capitata* L.). *Euphytica,* **184:** 265–273.

Singh, R. K., & Chaudhary, B. D. (1995). *Biometrical methods in quantitative genetic analysis*. Kalyani Publishers, New Delhi, India.

Sit, A. K., & Sirohi, P. S. (2000). Gene action of nutritional traits in bottle gourd [*Lagenaria Siceraria* (Mol.) Standl]. *Int. J. Veg. Sci.* ***27*(1):** 25-27.

Swarup, V. & Chatterjee, S. S. (1972). Origin and genetic improvement of Indian cauliflower. *Econ. Bot.* **26:** 381–393.

Tandon, H. L. S. (1998). *Micronutrients in soils, crops and fertilizers*. Fertilizer Development and Consultation Organization, New Delhi, India.

Thamburaj, S. & Singh, N. (2001). *Textbook of vegetables tuber crops and spices*. ICAR, New Delhi.

Verma, A. & Singh, Y. (2019). Estimation of genetic architecture of biochemical traits in mid–late cauliflower (*Brassica oleracea* L. var. *botrytis*) under sub-temperate conditions of north western Himalayas. *J. Genet. 98*, 24.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2022.00036.2.09

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Phenotypic Characterization and Diversity Analysis of a Collection of *Gladiolus* Cultivars in India using Morpho-physiological Traits

Varun M. Hiremath*, Kanwar P. Singh, Kishan Swaroop and Pooja Anand

Abstract

Gladiolus is a beautiful ornamental geophyte valued for varieties with attractive florets and various colors. The genetic diversity of *Gladiolus* is tremendous due to species richness, outcrossing nature and heterozygosity. In the current study, phenotypic characterization and diversity assessment of 84 Indian and exotic-bred genotypes were investigated. Among different quantitative and qualitative traits, the coefficient of variation varied from 7.90% (androecium length) to 48.23% (chlorophyll 'b'). All qualitative characteristics were polymorphic. In correlation analysis, 136 out of 253 pairwise inter-trait combinations revealed significant and positive correlations, although a significant and negative connection was found between the duration of flowering and the time of the beginning of flowering. Indirect selection for plant height, rachis length, and corm fresh weight may aid in the development of the linked traits. Principal component analysis revealed that six out of the 17 principal component axis had Eigen-values greater than one, accounting for 79.31% of the overall variability. The stable expression and better performance of *Gladiolus* genotypes for quantitative traits, such as plant height, length of the leaf blade, corm fresh weight, floret length, tepal length, and floret width would be useful in selection of superior offspring from hybridization. Cluster analysis divided whole germplasm into two major clusters: cluster I (29 genotypes) and cluster II (55 genotypes). Genotypes observed in subcluster Ia (08 genotypes) and subcluster IIc (13 genotypes) are relatively dissimilar from one another. Cultivar' Victor' is found to be relatively distinct among all genotypes because of taller plants, bigger corms, short flowering duration, and maximum corm fresh weight. Therefore, these set of genotypes can serve as a reference collection for DUS testing of novel varieties and genotype-specific information generated from this study can be useful for breeders to claim protection for their varieties

Keywords: Characterization, Corm, Descriptors, Diversity, *Gladiolus*.

Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi, Delhi, India.

***Author for correspondence:**

varunhiremath1992@gmail.com

Received: 29/08/2022 **Revised:** 23/01/2023

Accepted: 03/04/2023

How to cite this article: Hiremath, V.M., Singh, K.P., Swaroop, K., Anand, P. (2023). Phenotypic Characterization and Diversity Analysis of a Collection of *Gladiolus* Cultivars in India using Morpho-physiological Traits. *Indian J. Plant Genetic Resources*. 36(2), 265-279.
DOI: 10.5958/0976-1926.2022.00036.2.09

Introduction

Gladiolus (*Gladiolus* × *grandiflorus* L.) is a significant member of the Iridaceae family. It is a high-value bulbous flower crop grown all over the world for its eye-catching multi-colored spikes with long vase life. *Gladiolus* has 270 species (Goldblatt *et al*., 2014), with around 263 species found in Sub-Saharan Africa and Madagascar, seven species endemic to Eurasia (Goldblatt & Manning, 2008), about 170 species native to Southern Africa, and 93 species unique to tropical Africa. According to *Statista*, an international organization located in Germany, *Gladiolus* ranks fifth in terms of area and production among bulbous flowers grown worldwide (Pas, 2018). *Gladiolus* is grown on an estimated 11.66 thousand hectares in India, with a production of 106 crore cut flowers (agricoop.nic.in). *Gladiolus* is mostly a winter flower crop, but it may be produced throughout the year in mild climates. Its cut flowers are used to decorate vases, prepare bouquets, and for flower arrangements.

Gladiolus is a highly heterozygous, cross-pollinated crop. Its cultivars are said to have evolved naturally by

hybridization between the wild species *Gladiolus cruentus*, *G. natalensis*, *G. oppositiflorus*, *G. papilio* and *G. saundersii* (Imanishi, 1989). Because *Gladiolus* species are very cross-compatible, interspecific hybridization has become an important breeding strategy. Most species of *Gladiolus* have an identical basic chromosome number of x = 15 (Sinha and Roy, 2002) and a similar chromosomal configuration. Therefore, it became a popular plant for hybridization and the development of new colorful and vigorous hybrids; these new hybrids are estimated to number over 10,000 (Sinha and Roy, 2002). Flower patterns, floral colors, and flowering behavior of cultivated species vary greatly due to decades of interspecific hybridization and selection. New fascinating cultivars with novel floret color, vase life, pest and disease resistance are released each year to meet consumer demand.

Gladiolus is an exotic commercial crop in India with no native *Gladiolus* species found yet (Cantor and Tolety, 2011). *Gladiolus* hybrids and varieties have been routinely brought to India from South Africa and Europe since the 18th century for breeding and cultivation. Many *Gladiolus* hybrids and variants were subsequently developed utilizing these exotic varieties and released for cultivation in India. Systematic characterization involves distinguishing plant genetic resources by virtue of any difference in their phenotype or genetic make-up (De Vicente *et al.*, 2006). Genetically diverse germplasm broadens the genetic base by selecting distinct cross combinations in the hybridization programme. Accurately identifying and documenting cultivars is also important to protect the plant breeder's rights. Characterization data is used to monitor germplasm behavior, identify and distinguish between different accessions conserved in field or *in-vitro* gene banks. In this direction, different workers have characterized selected germplasm and delineated genetic diversity in previous studies in *Gladiolus* (Kadam *et al.,* 2014; Singh *et al*., 2018). Szczepaniak *et al.* (2016) have even clearly differentiated *G. palustris* and *G. imbricatus* using phenotypic traits (plant height, flower number, leaf width, leaf number and corm morphology) to find out the possibility of natural hybridization. Despite the importance of genus *Gladiolus*, research in India is still limited and comprehensive genetic diversity studies covering a wide range of accessions are inadequate. The objective of the study was to phenotypically characterize and assess genetic diversity of 84 popular cultivars of *Gladiolus* grown in India by using morpho-physiological descriptors.

Materials and Methods

Plant Material and Data Collection

A total of 84 diverse *Gladiolus* genotypes collected from various sources were used in the present study (Table 1). Commercially cultivated varieties by farmers in India include mostly exotic varieties. Characterization of these genotypes based on 35 morpho-physiological descriptors was carried out at the research farm of Division of Floriculture and Landscaping, IARI, New Delhi. It is situated at 77° 12' E longitude, 28°40' latitude and at an altitude of 228.16 m above mean sea level. *Gladiolus* corms (4–5 cm diameter) of respective genotypes were planted in last week of October at a spacing of 30 cm × 30 cm in randomized block design (RBD) with three replications under open field conditions during 2017-18 and 2018-19. Standardized uniform cultivation practices were followed throughout the cropping season to raise a good crop. DUS descriptors for all varieties, hybrids and parental lines of *Gladiolus* spp. and its cultivars developed by the Protection of Plant Varieties and Farmers' Rights Authority, New Delhi were used as reference guide for data collection (Kalloo *et al*., 2013). As mentioned in Tables 2 and 3, observations on 23 quantitative and 12 qualitative characteristics were recorded when the plants were at full flowering or expression stage. Data were recorded on ten randomly selected plants in each replication, avoiding border plants from each genotype.

Table 1: Indian and exotic *Gladiolus* cultivars used in the study.

Origin	*Institute/ organization*	*Cultivars*
Indian	BHU, Varanasi	Malaviya Shatabdi, Malaviya Kiran, Malaviya Kundan
	NBRI, Lucknow	Kalima, Neelima, Roshni, Mohini
	IARI, New Delhi	Pusa Gunjan, GS-2, Anjali, Dhanvantari, Pusa Shagun, Pusa Shabnam, Pusa Lohit, Pusa Manmohak, Pusa Sindhuri, Pusa Sringarika, Pusa Red Valentine, Neel Rekha, Pusa Suhagin, Pusa Urmi, Pusa Chirag, Pusa Archana, Pusa Sarang, Urmil, Delhi Callianthus, Pusa Urvashi, Pusa Chandni, Suchitra, Surya Kiran, Pusa Shweta, Pusa Srijana, Pusa Gulal, Pusa Kiran, Pusa Bindiya
	IIHR, Bengaluru	Arka Tilak, Arka Nazrana, Arka Aayush, Arka Sapna, Arka Kesar, Arka Amar, Arka Poonam, Arka Gold, Arka Aarti, Arka Naveen, Arka Darshan
	PAU, Ludhiana	Punjab Glad 1, Punjab Morning, Punjab Glance, Punjab Glad 2, Punjab Dawn, Punjab Lemon Delight, Punjab Pink Elegance, C.P.G
Exotic	-	Yellow Star, Amethyst, Aldebaran, Tiger Flame, Nova Lux, Euro Vision, Nicole, Fidelio, True Love, Vink's Glory, Anglia, Sancerre, Victor, Creamy Green, Friendship, Pink Lady, Plumtart, Lucky Shamrock, Peter Pear's, Vicki Lin, African Star, Apple Blossom, Summer Sunshine, Algarve, Fire Flame, Lady Jane, Yellow Stone, Rose Supreme, Jacksonville Gold, Praha

Table 2: Descriptive statistics for morpho-physiological characteristics of 84 *Gladiolus* genotypes evaluated at Delhi during 2017-18 and 2018-19

Trait	*Min.*	*Max.*	*Mean*	*SE*	*CV*	*Skewness*	*Kurtosis*
Plant height (cm)	40.42	78.00	57.73	0.84	13.32	0.42	2.86
Length of the leaf blade (cm)	30.33	62.25	43.70	0.64	13.38	0.66	3.75
Width of the leaf blade (cm)	1.81	4.30	3.01	0.06	17.17	0.33	2.72
Rachis length (cm)	18.50	52.25	35.35	0.72	18.79	0.34	2.90
Number of florets per spike (No,)	7.33	18.17	13.10	0.23	16.25	0.03	2.83
Bract length (cm)	3.47	7.03	4.90	0.09	16.77	0.43	2.64
Floret length (cm)	7.07	11.17	9.40	0.08	8.09	-0.16	3.14
Floret width (cm)	6.77	11.13	8.76	0.09	9.54	-0.05	3.06
Tepal length (cm)	4.53	8.47	5.96	0.07	11.34	1.05	5.61
Tepal width (cm)	2.50	5.70	3.97	0.07	15.62	0.55	3.18
Androecium length (cm)	3.78	5.65	4.63	0.04	7.90	0.25	2.98
Anther length (cm)	0.53	1.78	1.27	0.02	16.12	-0.68	4.77
Stigma lobe length (cm)	0.47	1.37	0.71	0.02	24.93	1.38	5.56
Style length (cm)	3.88	7.47	5.96	0.06	9.09	-0.26	5.06
Time of beginning of flowering (days)	72.33	122.50	104.39	1.15	10.12	-0.62	2.91
Duration of flowering (days)	6.83	36.50	14.65	0.60	37.28	1.23	5.12
Corm diameter (days)	3.92	6.88	5.36	0.07	12.30	-0.12	2.56
Corm fresh weight (g)	23.08	90.83	44.64	1.37	28.04	0.63	4.06
Chlorophyll 'a' (mg/g)	1.28	6.06	4.04	0.10	22.73	-0.18	3.07
Chlorophyll 'b' (mg/g)	0.03	1.77	0.62	0.03	48.23	1.14	4.90
Total chlorophyll (mg/g)	1.27	7.33	4.65	0.12	23.44	-0.09	3.24
Total carotenoids (mg/g)	0.69	2.11	1.51	0.03	18.27	-0.18	3.03
Normal difference vegetation index	0.43	0.66	0.53	0.01	9.50	0.44	2.94

Qualitative data were collected per DUS guidelines and described with characteristics and their states. Floret color and corm skin color were recorded during day time (11.00 AM to 1.00 PM) using Royal Horticultural Society (RHS) color chart (6th edition, 2015) as per the guidelines recommended by the International Union for the Protection of New Varieties of Plants (UPOV), Geneva, Switzerland.

Physiological characteristics like leaf chlorophyll content (mg/g FW) was estimated by a non-maceration method using dimethyl sulphoxide (DMSO) as described by Hiscox and Israelstam (1979). A total of 50 mg of finely chopped leaf samples were placed in test tubes to which 10 mL DMSO was added. The test tubes were covered with aluminum foil and kept in an oven at 65°C for 4 hours. After 4 hours, the absorbance of chlorophyll solution was recorded at 470, 645 and 663 nm against DMSO as blank. Chlorophyll 'a', 'b' and total chlorophyll were calculated by using the formulae chlorophyll 'a' = 12.7 A_{663} - 2.69 A_{645}; chlorophyll 'b' = 22.9 A_{645} - 4.68 A_{663}, where 'A' is OD value (Arnon, 1949). The total carotenoid content (mg/g FW) was calculated as per the formulae total carotenoids = [1000 A_{470} - (3.27 Chlorophyll 'a' + 104 Chlorophyll 'b')]/229, where 'A' is OD value (Lichtenthaler and Wellburn, 1983). Normalized Difference Vegetation Index was recorded on random group of plants in each plot using 'GreenSeeker' NDVI meter to estimate the relative chlorophyll content in each *Gladiolus* genotype.

Statistical Analysis

Morpho-physiological data recorded during two consecutive years of 2017-18 and 2018-19 was subjected to pooled analysis and mean values of each character for all *Gladiolus* genotypes were calculated. The recorded data for quantitative characters were analyzed using the software package R of version 4.2.0 developed by the R Core Team (2022). Descriptive statistics, including the mean, median, range, variance, skewness and kurtosis were calculated for all quantitative variables. Coefficients of variation [CV = (Std. Dev./Mean) *100] were estimated as the indicator of variability. Box plots for quantitative characters were created using the "ggplot2" package. The Pearson correlation coefficients (r) among the 23 different qualitative traits were estimated using the package "Corrplot". Principal component analysis (PCA) was done to determine the extent of the variation and percentage similarity within the *Gladiolus* genotypes. The Euclidean distance was used to calculate the distance coefficients matrix for cluster analysis using UPGMA method. The dendrogram was constructed using the "*factoextra*" R package.

Table 3: Twelve qualitative characteristics and their states in 84 *Gladiolus* genotypes evaluated at Delhi during 2017-18 and 2018-19

Cv. No.	*Cultivar name*	*Leaf blade color*	*Anthocyanin coloration of bracts*	*Shape of apex of bracts*	*Floret color**	*Flower secondary color*	*Outer skin color of corm#*	*Outer whorl shape*	*Filament main color*	*Stigma lobe color*	*Floret shape*	*Flower attitude*	*Arrangement of florets on spike@*
1	Anglia	DG	W	O	Fan1, Yellow group 8(B)	Yellow	Yellowish -Orange	O	Light yellow	Yellow	T	U	T
2	Amethyst	YG	W	A	Fan 2, Purple - Violet group N80 (C)	White (pure)	Light yellow	O	Light pink	Pink purple	T	SU	Z
3	Pusa Gunjan	DG	W	O	Fan1, Orange group 27(A)	Red	Dark yellow	E	Light pink	White	S	SU	Z
4	Punjab Glad 1	LG	W	BO	Fan1, Orange-Red Group 33(D)	Yellow	Dark yellow	O	Light yellow	Pink purple	T	SU	Z
5	Aldebaran	DG	W	A	Fan1, Yellow group 9(D)	Red	Dark yellow	O	Light yellow	Yellow	S	SU	Z
6	Fire Flame	LG	A	O	Fan 1, Red group (45B)	Yellow	Yellowish -orange	E	White	Pink purple	T	SU	Z
7	Tiger Flame	DG	W	O	Fan 1, Red Group 38(A)	Yellow	Dark yellow	E	Light yellow	Yellow	T	SU	T
8	Kalima	LG	S	O	Fan 1, Red group (45B)	None	Purple	O	Light yellow	Pink purple	T	SU	Z
9	Neelima	YG	W	A	Fan 2, Purple group 77(B)	White (pure)	Light yellow	O	White	Blue purple	S	U	T
10	GS-2	DG	W	BO	Fan 1, Yellow group 4(D)	Red	Light yellow	E	Light yellow	Yellow	R	SU	O
11	Praha	DG	M	A	Fan 1, Orange Red Group N30(A)	Yellow	Purple	O	Light yellow	Red	T	SU	T
12	Anjali	LG	W	O	Fan 4, White group NN155(B)	Pink	Dark yellow	O	White	White	R	SU	Z
13	Malaviya Shatabdi	LG	W	O	Fan 2, Red purple group 69 (C)	Pink	Yellowish -orange	O	White	White	T	U	T
14	Roshni	DG	M	A	Fan 1, Red group 43(B)	Yellowish-green	Orange	E	Light pink	Pink purple	T	U	Z
15	Yellow Stone	YG	W	O	Fan 1, Yellow group 8(C)	None	Orange	E	Light yellow	Yellow	T	SU	T
16	Dhanvantari	LG	W	A	Fan 1, Yellow group 4(D)	Red	Yellowish -orange	O	Light yellow	Yellow	S	SU	Z

17	Punjab Morning	DG	W	O	Fan 2, Red purple group 65(C)	Red	Yellowish -orange	O	White	White	T	U	T
18	Rose Supreme	DG	W	A	Fan 1, Red Group 48(D)	Yellowish-green	Light yellow	O	Light yellow	White	T	U	T
19	Punjab Glance	DG	W	A	Fan 1, Red Group 38(A)	Yellow	Light yellow	O	White	White	T	U	Z
20	Sancerre	DG	W	A	Fan 4, White group NN155(B)	Pink	Light yellow	E	White	White	S	SU	Z
21	Pusa Shagun	LG	M	O	Fan 1, Orange Red Group N30(B)	Purple red	Purple	O	Light pink	Red	T	SU	T
22	Punjab Glad 2	LG	A	A	Fan 1, Yellow group 8(D)	Pink	Light yellow	Ob	Light yellow	Pink purple	S	SU	T
23	Pusa Shabnam	DG	M	O	Fan 1, Yellow group 8(D)	Pink	Yellowish -orange	E	White	White	T	U	T
24	Punjab Dawn	DG	W	O	Fan 1, Red Group 38(A)	Red	Orange	O	White	White	T	SU	T
25	Arka Tilak	DG	M	O	Fan 1, Orange red group N30A	None	Red	E	Light red	Red	T	U	Z
26	Victor	DG	W	O	Fan 3, Yellow Green 154(D)	None	Yellowish -orange	E	Light yellow	Yellow	S	SU	T
27	Pusa Lohit	LG	S	O	Fan 1, Red group 44(D)	Pink	Red	E	Light pink	Pink purple	S	U	Z
28	Malaviya Kiran	LG	M	A	Fan 4, White group NN155(A)	Purple red	Yellowish -orange	E	White	White	T	U	T
29	Pusa Manmohak	DG	M	O	Fan 1, Red group (40B)	Red	Red	O	Light pink	Red	S	SU	T
30	Pusa Sindhuri	DG	M	BO	Fan 1, Red group 51(A)	Yellowish-green	Light yellow	E	Light yellow	Pink purple	S	SU	T
31	Arka Nazrana	DG	VS	A	Fan 2, Red purple group 61(B)	None	Purple	O	White	Pink purple	S	U	T
32	Nova Lux	DG	W	A	Fan1, Yellow group 11(B)	Purple red	Light yellow	E	Light yellow	Yellow	T	U	T
33	Euro Vision	DG	S	A	Fan 1, Red group 40(A)	None	Purple	Ob	Light yellow	Pink purple	T	SU	Z
34	Lady Jane	LG	M	BO	Fan 1, Red group 41(B)	Yellow	Orange	O	Light yellow	Yellow	T	SU	Z

35	Jacksonville Gold	YG	W	A	Fan1, Yellow group 6(D)	Red	Light yellow	E	Light yellow	Yellow	T	U	T
36	Punjab Lemon Delight	YG	M	BO	Fan 1, Yellow group 8(D)	Pink	Light yellow	O	Light yellow	Yellow	S	U	Z
37	Arka Aayush	LG	S	BO	Fan 1, Red group 41(B)	Yellow	Dark yellow	E	Light yellow	Yellow	T	SU	T
38	Pusa Sringarika	DG	W	O	Fan 2, Pink group NN 74(B)	Yellow	Light yellow	E	White	Yellow	T	SU	T
39	Pusa Red Valentine	DG	M	BO	Fan 1, Red group 45 (C)	Pink	Red	Ob	Medium pink	Pink purple	T	SU	Z
40	Neel Rekha	DG	M	O	Fan 2, Red-purple group 64 (B)	None	Orange	O	Light pink	Blue purple	S	SU	Z
41	Yellow Star	DG	W	O	Fan 1, Yellow group 10(B)	None	Light yellow	E	Light yellow	Yellow	R	U	Z
42	Creamy Green	LG	W	A	Fan 4, White group NN155(D)	None	Light yellow	O	White	White	T	U	Z
43	Friendship	YG	W	A	Fan 4, White group NN155(D)	None	Dark yellow	Ob	White	White	R	SU	T
44	Pink Lady	DG	W	A	Fan 1, Red group 49 (C)	Orange	Dark yellow	E	Light pink	White	R	SU	Z
45	Arka Sapna	DG	W	O	Fan 4, White group 155(B)	Yellowish-green	Dark yellow	E	White	Pink purple	S	SU	T
46	Arka Kesar	LG	W	BO	Fan 1, Orange group 29(B)	Orange	Light yellow	E	Light yellow	Yellow	T	U	Z
47	Pusa Suhagin	DG	S	A	Fan 1, Red group 46(A)	None	Red	E	Light red	Red	T	SU	Z
48	Pusa Urmi	DG	W	O	Fan 2, Pink group NN74(A)	Violet blue	Orange	E	Light pink	Blue purple	T	SU	Z
49	Pusa Chirag	DG	W	BO	Fan 1, Red group 41(C)	Yellow	Light yellow	O	Light pink	Yellow	T	U	Z
50	Nicole	YG	M	O	Fan 1, Orange red 30(A)	Yellow	Purple	E	Light yellow	Pink purple	T	U	Z
51	Pusa Archana	YG	W	BO	Fan 2, Red purple 68(B)	Yellowish-green	Purple	O	White	Pink purple	S	SU	Z
52	Arka Darshan	DG	M	BO	Fan 2, Red purple 63(B)	White (pure)	Orange red	E	Light pink	Pink purple	T	U	T

53	Plumtart	LG	S	A	Fan 2, Red purple 71(B)	None	Red	E	Medium pink	Violet	S	SU	Z
54	Pusa Sarang	DG	S	O	Fan 2, Purple group 76(A)	White (pure)	Purple red	E	Medium pink	Blue purple	T	SU	T
55	Fidelio	DG	W	O	Fan 2, Purple-violet group N80(A)	None	Orange	O	White	Blue purple	T	SU	T
56	Urmil	DG	W	A	Fan 2, Purple-violet group N80(B)	White (pure)	Orange	E	Light yellow	Blue purple	T	SU	Z
57	Lucky Shamrock	DG	W	O	Fan 1, Orange red group 33(D)	Orange	Orange	E	Light pink	Orange	T	SU	Z
58	Delhi Callianthus	LG	W	O	Fan 1, Red group 43(C)	Pink	Dark yellow	E	White	Orange	T	SU	Z
59	Pusa Urvashi	DG	M	O	Fan 2, Red purple 70(A)	White (pure)	Yellowish -orange	E	White	Blue purple	T	SU	T
60	Pusa Chandni	DG	M	A	Fan 1, Yellow group 11(D)	Yellow	Yellowish -orange	E	White	White	S	U	T
61	Suchitra	DG	W	A	Fan 1, Red group 56(B)	Red	Light yellow	O	White	White	S	U	T
62	Surya Kiran	YG	W	A	Fan 1, Red group 37(B)	Red	Yellowish -orange	E	White	White	T	SU	T
63	Arka Amar	LG	VS	O	Fan 1, Red group 48(B)	Yellow	Red	E	Light pink	Red	T	U	T
64	True Love	DG	M	O	Fan 2, Red purple N66(C)	White (pure)	Orange	E	Light pink	Pink purple	S	SU	O
65	Peter Pear's	LG	W	A	Fan 1, Orange Red group 32(C)	White (pure)	Orange	E	White	White	T	SU	T
66	Pusa Shweta	DG	W	O	Fan 4, White group 155(B)	None	Dark yellow	O	Light yellow	White	T	U	T
67	Arka Poonam	YG	W	O	Fan 4, White group 155(A)	None	Others	E	White	White	T	U	Z
68	Pusa Srijana	DG	VS	A	Fan 2, Red purple group 71(C)	Yellow	Yellowish -orange	O	Light pink	Blue purple	S	U	Z
69	Pusa Gulal	DG	M	O	Fan 2, Red purple 61(B)	None	Red	E	Medium pink	Pink purple	S	SU	T
70	Vicki Lin	DG	W	A	Fan 1, Orange red group 33(D)	Orange	Orange	O	Light pink	Orange	T	H	Z

71	African Star	DG	W	O	Fan 1, Red group 48(C)	Red	Light yellow	O	White	White	T	U	Z	
72	Arka Gold	DG	W	BO	Fan 1, Yellow group 8(D)	Red	Others	O	Light yellow	Yellow	T	SU	Z	
73	Pusa Kiran	YG	M	O	Fan 1, Yellow group 11(D)	Pink	Yellowish -orange	E	White	White	R	SU	Z	
74	Arka Aarti	DG	VS	A	Fan 1, Red group 44(B)	Red	Yellowish -orange	E	Light pink	Red	T	SU	Z	
75	Arka Naveen	YG	A	O	Fan 2, Violet group 84 (C)	Yellow	Light yellow	O	White	White	T	U	Z	
76	Apple Blossom	DG	A	O	Fan 1, Yellow group 11(D)	Purple Red	Light yellow	O	White	Blue purple	T	U	Z	
77	Punjab Pink Elegance	LG	W	A	Fan 1, Red group 56 (D)	Red	Light yellow	E	White	White	T	U	T	
78	Vink's Glory	DG	W	O	Fan 1, Yellow group 8(C)	None	Light yellow	E	Light yellow	Yellow	T	U	Z	
79	Pusa Bindiya	YG	W	O	Fan 1, Red group 36(D)	Red	Light yellow	O	White	White	T	U	Z	
80	Pusa Mohini	DG	W	O	Fan 2, Pink group NN74(C)	White (Pure)	Light yellow	E	White	White	S	U	Z	
81	Summer Sunshine	LG	A	A	Fan 1, Yellow group 6(C)	None	Dark yellow	E	Light yellow	Yellow	T	SU	Z	
82	Algarve	DG	M	O	Fan 1, Orange group 25(B)	None	Light yellow orange	E	Light yellow	Pink purple	S	SU	T	
83	Malaviya Kundan	YG	W	BO	Fan 1, Yellow orange 18(B)	Yellowish-Green	Light yellow	O	Light yellow	Yellow	T	SU	Z	
84	C.P.G	YG	S	A	Fan 1, Red group 46(A)	None	Red	O	Light red	Red	T	SU	Z	

LG=Light green, YG=Yellowish green, DG= Dark green

A= Absent, W =Weak, M= Medium S= Strong VS=Very Strong

A= Acute O=Obtuse O=Ovate BO=Broadly Obtuse

*Refer Figure 3

#Refer Figure 4

E= Elliptic O= Ovate Ob= Obovate

T= Triangular S= Star R= Round

U=Upright SU=Semi-uptight H= Horizontal

T=Two rows Z=Zig-Zag O=One row

@Figure 2

Table 4: Eigen values, proportion of variability and factor loadings (Eigen vectors) of correlation matrix for 17 morpho-physiological traits of 84 *Gladiolus* genotypes evaluated at Delhi during 2017-18 and 2018-19

Characters	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16	PC17
Plant height	0.36	0.13	0.21	0.12	0.34	-0.22	0.08	-0.19	0.26	0.14	0.02	0.06	-0.16	-0.23	-0.44	0.48	0.02
Length of the leaf blade	0.36	0.14	0.17	0.13	0.37	-0.18	0.06	-0.16	0.35	-0.13	0.08	0.03	0.16	0.35	0.36	-0.43	-0.01
Rachis length	-0.01	0.08	-0.34	0.49	0.14	0.24	0.19	0.09	0.01	-0.04	0.36	-0.12	0.46	-0.36	-0.14	-0.12	-0.01
Number of florets per spike	-0.10	0.00	-0.28	0.49	0.18	0.39	0.12	0.00	0.06	-0.04	-0.27	-0.02	-0.45	0.42	0.01	0.16	0.01
Bract length	0.20	0.24	-0.16	-0.11	0.09	0.23	-0.61	-0.42	-0.21	-0.02	0.03	-0.46	0.02	0.04	-0.05	0.02	-0.02
Floret length	0.22	0.42	-0.17	-0.06	-0.15	0.06	-0.01	-0.04	-0.23	-0.54	-0.06	0.58	0.06	0.04	-0.14	0.05	0.01
Floret width	0.24	0.37	-0.14	-0.03	-0.26	-0.08	0.26	0.18	0.16	0.02	-0.59	-0.39	0.12	-0.20	0.15	0.03	0.02
Tepal length	0.23	0.31	-0.27	0.00	-0.20	-0.29	0.16	0.18	-0.22	0.30	0.50	-0.10	-0.42	0.13	0.07	-0.09	-0.02
Time of beginning of flowering	0.05	0.17	-0.25	-0.38	0.38	0.30	-0.05	0.23	0.05	0.56	-0.12	0.34	0.18	0.05	0.03	-0.01	0.00
Duration of flowering	-0.08	0.09	0.00	0.49	-0.33	-0.25	-0.30	-0.29	-0.03	0.46	-0.21	0.33	0.17	0.01	0.08	-0.05	-0.01
Corm diameter	0.30	-0.07	0.39	0.13	-0.09	0.13	0.14	0.23	-0.44	0.13	-0.02	-0.14	0.37	0.46	-0.23	0.13	-0.02
Corm fresh weight	0.27	-0.01	0.41	0.16	0.07	0.36	0.00	0.05	-0.31	0.07	-0.06	0.12	-0.33	-0.48	0.31	-0.21	0.01
Chlorophyll 'a'	0.32	-0.38	-0.24	0.01	-0.02	-0.10	-0.11	0.10	0.01	-0.02	-0.19	0.04	-0.10	-0.05	-0.29	-0.30	-0.66
Chlorophyll 'b'	0.18	-0.26	-0.18	-0.17	-0.15	0.16	0.46	-0.59	-0.10	0.10	0.08	0.06	0.11	0.01	0.31	0.26	-0.18
Total chlorophyll	0.32	-0.39	-0.25	-0.04	-0.06	-0.03	0.03	-0.08	-0.03	0.03	-0.11	0.04	-0.05	-0.01	-0.23	-0.28	0.72
Total carotenoids	0.30	-0.30	-0.20	0.12	0.02	-0.13	-0.36	0.37	0.04	-0.13	0.09	0.05	0.10	-0.04	0.46	0.48	0.06
NDVI	0.19	0.02	0.16	-0.04	-0.52	0.46	-0.13	0.07	0.58	0.06	0.25	0.06	-0.04	0.08	-0.11	0.00	-0.01
Eigen value	3.92	2.84	2.35	2.06	1.20	1.12	0.87	0.81	0.55	0.37	0.31	0.18	0.16	0.13	0.09	0.06	0.00
%Variance	23.06	16.72	13.79	12.12	7.04	6.58	5.10	4.78	3.22	2.16	1.79	1.05	0.96	0.76	0.53	0.33	0.01
%Cumulative variance	23.06	39.77	53.57	65.69	72.73	79.32	84.41	89.19	92.41	94.56	96.36	97.40	98.36	99.12	99.65	99.99	100.00

Table 5: Cluster wise means for different quantitative traits of 84 *Gladiolus* genotypes evaluated at Delhi during 2017-18 and 2018-19.

	Characters		*PH*	*LLB*	*RL*	*NFS*	*BL*	*FL*	*FW*	*TL*	*TBF*	*DF*	*CD*	*CFW*	*Chl 'a'*	*Chl 'b'*	*Total Chlorophyll*	*Total carotenoids*	*NDVI*
Major Cluster I	Sub-cluster Ia	Mean	69.12	52.36	30.02	11.19	5.95	10.03	9.04	6.11	109.54	13.19	6.11	63.85	3.90	0.46	4.36	1.52	0.57
		±SE	2.44	1.87	1.46	0.70	0.27	0.27	0.25	0.17	2.51	1.85	0.21	5.10	0.19	0.03	0.19	0.05	0.02
	Sub-cluster Ib	Mean	60.97	46.44	39.24	14.00	5.09	9.63	9.08	6.34	104.56	14.55	5.57	46.84	4.93	0.85	5.78	1.74	0.54
		±SE	1.06	0.75	1.60	0.50	0.18	0.11	0.14	0.16	2.23	1.01	0.12	1.77	0.12	0.08	0.14	0.05	0.01
Major Cluster II	Sub-cluster IIa	Mean	53.57	41.89	38.07	13.70	5.03	9.85	9.26	6.31	114.32	14.86	4.80	34.31	2.91	0.47	3.39	1.23	0.52
		±SE	2.52	2.19	1.42	0.39	0.17	0.22	0.31	0.16	1.68	0.98	0.12	2.57	0.22	0.07	0.26	0.07	0.01
	Sub-cluster IIb	Mean	54.48	40.44	30.17	11.89	4.70	8.92	8.29	5.48	107.01	11.07	4.91	36.09	4.41	0.71	5.08	1.54	0.51
		±SE	1.31	0.93	1.12	0.42	0.20	0.17	0.18	0.09	1.61	0.59	0.15	2.17	0.18	0.07	0.20	0.06	0.01
	Sub-cluster IIc	Mean	55.74	41.78	35.90	13.48	4.47	9.12	8.46	5.74	95.67	17.52	5.53	47.82	3.68	0.50	4.16	1.44	0.53
		±SE	1.22	0.76	1.09	0.39	0.11	0.13	0.14	0.12	1.89	1.39	0.09	1.82	0.10	0.04	0.13	0.04	0.01

Results and Discussion

Descriptive Characteristics

The data pertaining to the variation among 84 *Gladiolus* genotypes for morpho-physiological characters are given in Table 2. The coefficient of variation varied from 7.90% (androecium length) to 48.23% (chlorophyll 'b'). Chlorophyll 'b' content (Chl' b') and duration of flowering (DF) showed high CV values of greater than 30%. Corm fresh weight (CFW), stigma lobe length (SLL), total chlorophyll (TCH), chlorophyll 'a' (Chl' a'), rachis length (RL), total carotenoids (TC), width of leaf blade (WLB), bract length (BL), number of florets per spike (NFS), anther length (ATL), tepal width (TW) showed moderate CV values ranging from 15 to 30%. Length of leaf blade (LLB), plant height (PH), corm diameter (CD), tepal length (TL), time of beginning of flowering (TBF), floret width (FW), normal difference vegetation index (NDVI), style length (SL), floret length (FL) and androecium length (AL) showed low CV values of less than 15%. In a similar line of study, Pattanaik *et al*. (2015) also reported the highest CV (>20%) for corm weight, rachis length and number of spikes per plant. CV is very important in selecting elite parents in the breeding of *Gladiolus* × *hybridus* (Hort *et al*., 2014). Skewness and kurtosis values indicated that morpho-physiological data distributed among all cultivars is symmetrical and follows a normal distribution. This variability in *Gladiolus* germplasm could be related to the high heterozygosity achieved during outcrossing.

Phenotypic characterization is the primary criteria for classifying and identifying crop species and varieties. It helps to obtain robust genetic information that would guide us in germplasm conservation. Vegetative and reproductive characteristics generally allow for quick genotype determination and genetic trait association (Datta and Chakrabarty, 2016). The expression of phenotypic features at the gene level allows for genetic differentiation of crops, and these traits become transmissible in subsequent generations (De Vicente *et al*., 2006). In the case of *Gladiolus*, it also aids in the identification of more reliable features other than commonly used criteria such as floret color, spike length, number of florets per spike, floret diameter, and vase life for the purpose of selecting superior genotypes in hybridization.

Box plots showing genetic variability among 84 *Gladiolus* genotypes with respect to various morpho-physiological characters and outliers have been depicted in Figure 1. In the present study, box plots displayed highly informative means suggesting considerable variation in selected *Gladiolus* germplasm. *Gladiolus* cultivars showing extreme variability were identified and plotted as outliers for morphological characters *viz*., length of leaf blade (Praha, Pusa Shagun), corm fresh weight (Victor), duration of flowering (Malaviya Shatabdi, Yellow Stone), floret

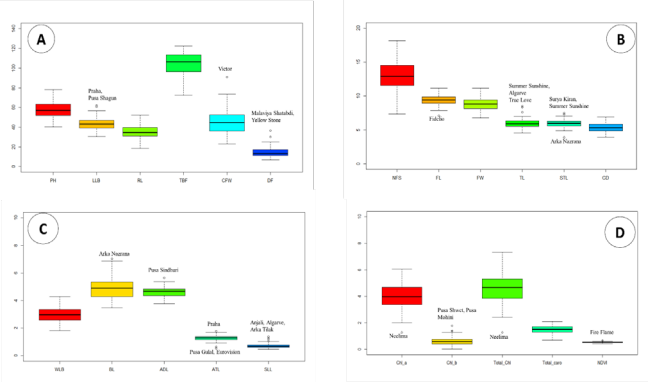

Figure 1: Box plots indicating genetic variability in quantitative characteristics in *Gladiolus* germplasm. Graph A - boxplots of plant height (PH), length of the leaf blade (LLB), rachis length (RL), time of beginning of flowering (TBF), corm fresh weight (CFW), duration of flowering (DF); Graph B – boxplots of number of florets per spike (NFS), flower length (FL), flower width (FW), tepal length (TL), stigma length (STL), corm diameter (CD); Graph C – boxplots of width of leaf blade (WLB), bract length (BL), androecium length (ADL), anther length (ATL), stigma lobe length (SLL); Graph D – boxplots of chlorophyll 'a' (Chl'a'), chlorophyll 'b' (Chl'b'), total chlorophyll (Total Chl), total carotenoids (Total caro), Normal Difference Vegetation Index (NDVI). For every box plot, the horizontal line represents the median of the trait, the box represents the interquartile range, the bars outside the box represent the extremes and the dots indicate the outliers

length (Fidelio), tepal length (True Love, Algarve, Summer Sunshine), stigma length (Surya Kiran, Summer Sunshine, Arka Nazrana), bract length (Arka Nazrana), androecium length (Pusa Sindhuri), anther length (Praha, Pusa Gulal, Euro Vision) and stigma lobe length (Anjali, Algarve, Arka Tilak). Kumar and Kulkarni (2009) assessed seven parents and their 21 hybrids using vegetative and flowering characteristics for phenotypic variation. It has been reported that days to sprouting, plant height, stem girth, number of leaves, leaf length, days required for bud initiation, days required for first floret opening and days required for first to last floret opening can be useful criteria for selection of better progenies subsequent to hybridization. For physiological characteristics such as chlorophyll 'a'(Neelima), chlorophyll 'b' (Pusa Shwet, Pusa Mohini), total chlorophyll (Neelima) and for NDVI (Fire Flame), varieties with extreme values were also detected. All qualitative characters are found to be polymorphic among all *Gladiolus* genotypes. States of each qualitative characteristic for all *Gladiolus* genotypes used in the study are given in Table 3. Spikes of selected *Gladiolus* genotypes are presented in Figure 2. Floret color and corm skin color are the most reliable morphological descriptors for differentiating *Gladiolus* varieties. It is feasible to employ them for the certification of genetic purity in *Gladiolus* cultivars. Outer corm skin color of selected *Gladiolus* genotypes is depicted in Figure 3.

Correlation Analysis

Information obtained by analyzing the coefficients of correlation between the plant traits helps us understand

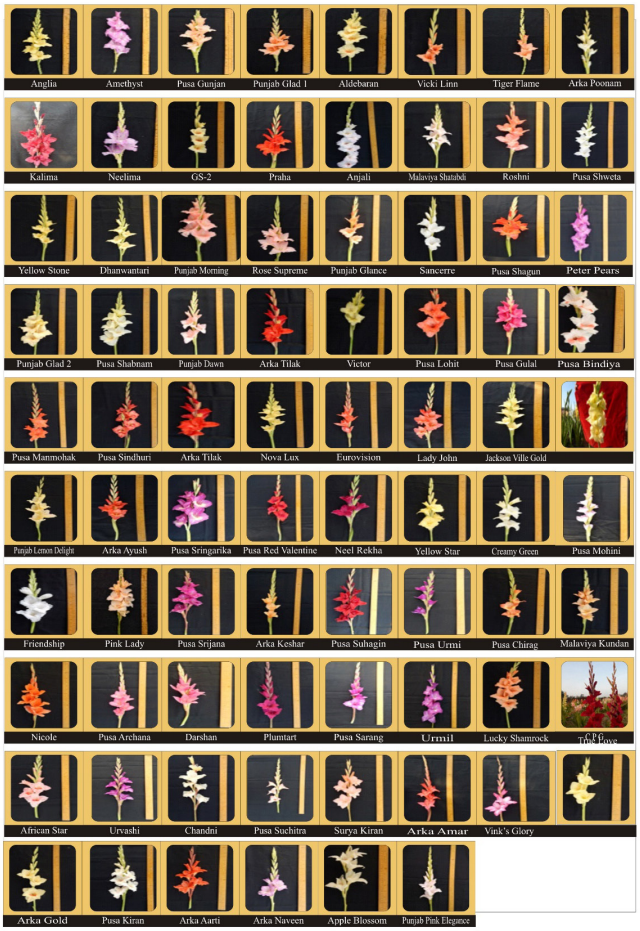

Figure 2: Spike characteristics of representative *Gladiolus* genotypes evaluated at Delhi during 2017-18 and 2018-19

each character's relative influence on flower quality and yield in *Gladiolus* production. Correlation coefficients between the morpho-physiological traits could determine whether selection for one trait may affect the other ones. In the present study, altogether positive and negative correlations were observed among twenty three different traits (Figure 4). The figure represents that the correlation coefficients with the absolute measures more than 0.22 and 0.28 were statistically significant at 5 and 1% probability, respectively. Meanwhile, significant correlations of 'r' value more than ±0.50 between the traits indicated relative influence of each character. Significant and positive correlations were obtained for plant height with length of leaf blade (r = 0.90) and corm fresh weight (r = 0.51); bract length and floret length (r = 0.54); floret width with floret length (r = 0.67) and tepal length (r = 0.69); rachis length with number of florets per spike (r = 0.78); corm diameter with corm fresh weight (r = 0.80). The relationship between plant height, length of leaf blade and corm fresh weight was apparent because length of leaf blade serves as a source of photosynthesis, directing photosynthates towards plant growth and eventually carbohydrate accumulation in corms. Correlations for floret characteristics *viz.*, bract length, floret

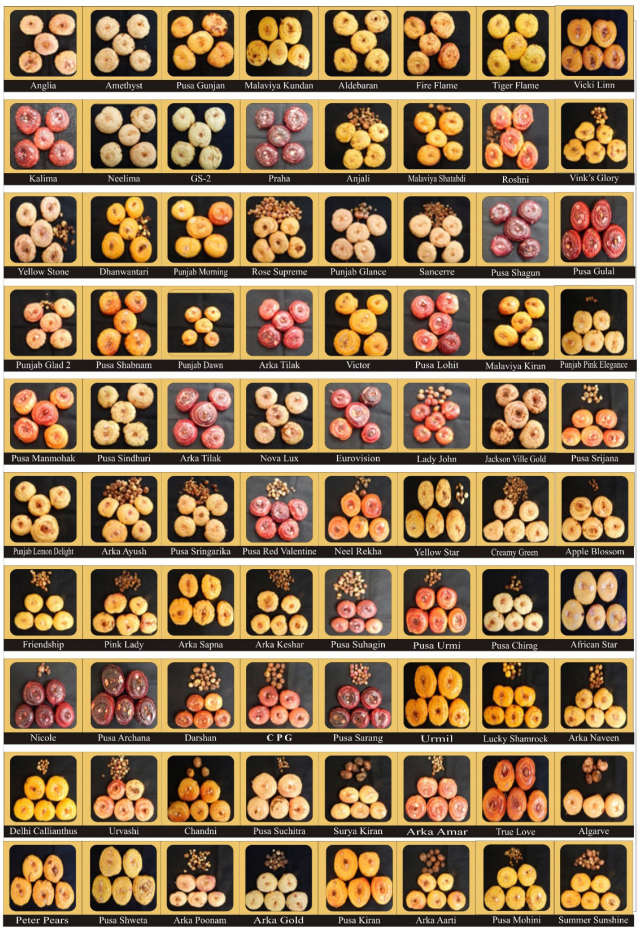

Figure 3: Corm skin color of representative *Gladiolus* genotypes evaluated at Delhi during 2017-18 and 2018-19

length, floret width and tepal length were found significant with each other. Rachis length exhibited significant and positive correlation with number of florets per spike. For corm characters, correlation was obvious between corm diameter and corm fresh weight. The correlations for these characters observed in our study were consistent with those reported earlier in *Gladiolus* by Chaudhary *et al*. (2011) and Zaharia *et al*. (2018). Furthermore, it is evident that a heavier corm and/or a larger corm size result in a higher plant height and rachis length. Among physiological characteristics, significant and positive correlations were found for total chlorophyll with chlorophyll 'a' (r = 0.97), chlorophyll 'b' (0.68) and total carotenoids (r = 0.75). This revealed a close relationship between the various chlorophyll components in leaves. Flowering duration had a significant but negative correlation with time taken to flowering (r = - 0.50). Duration of flowering may be longer as there is an increase in rachis length and the number of florets per spike. Since *Gladiolus* flower 3 to 4 months after the vegetative phase, they have a lengthy juvenile period. In light of this finding, our work hypothesizes that indirect selection for plant height, rachis length, and corm fresh weight may aid in the development of the linked traits. In the same line of study, Ramzan *et al*. (2016)

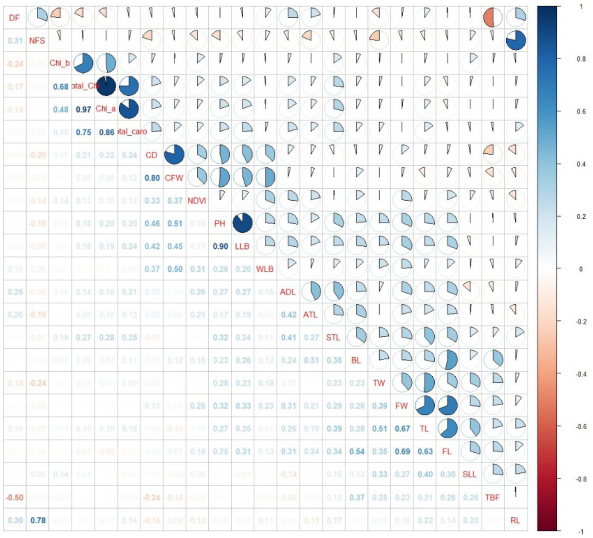

Figure 4: Pearson correlation coefficients among 23 quantitative traits of 84 *Gladiolus* genotypes evaluated during 2017-18 and 2018-19. The correlation coefficients with the absolute values more than 0.22 and 0.28 were significant at the statistical probability level of 5 and 1%, respectively

stated that indirect selection for floret breadth in hybrid progenies simultaneously improve number of florets per spike and rachis length due to direct and positive correlation between them. Among the 253 potential trait combinations, 136 pairwise inter-trait combinations revealed significant and positive correlations, although a significant and negative connection was found between flowering duration and time of beginning of flowering. Positive correlations between two attributes typically indicate that improving one trait will improve the other.

Principal Component Analysis

Principal component analysis (PCA) allows us to determine the traits that contribute the most to variability as well as recognize trait-specific germplasm for breeding. It is also a successful method of evaluating germplasm for desirable characteristics. In the current study, 17 morpho-physiological characters with significant correlations were used to create principal components that can explain most of the variance. Eigen-values, proportion of variability and factor scores obtained from PCA were used to determine the axis's relative discriminative power and associated characters (Table 4). Scree plot showing the contribution of each principal component towards variability in *Gladiolus* germplasm is presented in Figure 5. Table 4 reveals that six out of the 17 principal component axis had Eigen-values greater than one, and all together account for 79.31% of the total variability. The bold-marked values indicate the highest correlations between variables and the corresponding factor or principal component (PC). The first factor which accounted for 23.06% of the variation was strongly associated with plant height, length of leaf blade,

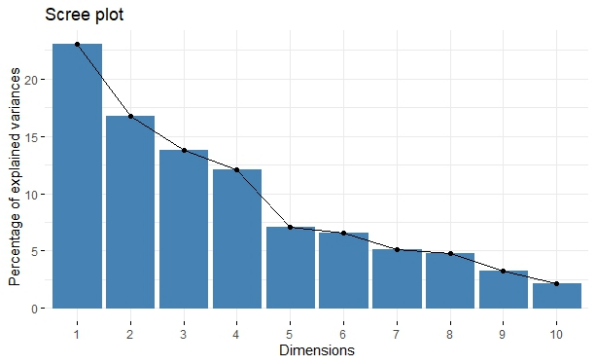

Figure 5: Scree plot showing contribution of each principal component towards variability of 84 *Gladiolus* genotypes evaluated during 2017-18 and 2018-19

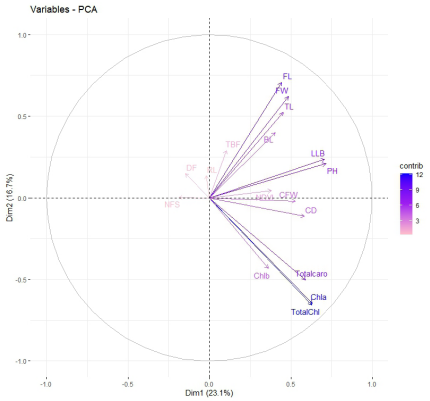

Figure 6: Variable correlation plot for seventeen quantitative traits of 84 *Gladiolus* genotypes evaluated during 2017-18 and 2018-19

bract length, floret length, floret width, tepal length, corm diameter, corm fresh weight, chlorophyll 'a', chlorophyll 'b', total chlorophyll, total carotenoids and normal difference vegetation index. PC 1 can thus be considered a component of productivity because it contains multiple characteristics that are components of production. A similar study on *Gladiolus* found that corm diameter, days to first floret opening and colour bud display, spike and stem diameter accounted for the greater part of variation in genotypes (Zahoor *et al*., 2019). The second factor which accounts for 16.72% of the variation, has affected bract length, floret length, floret width and tepal length. Positive loadings were found for all variables in the first and second factors. The third factor with significant positive loadings, accounting for 13.79% of total variation contributed by plant height, corm diameter and corm fresh weight. The variation accounted in the fourth factor (12.12%) was found to be significantly related to rachis length, number of florets per spike and flowering duration. The fifth factor (7.04%) consisted of variation contributed from traits plant height, length of leaf blade and time of beginning of flowering whereas the sixth factor was characterized by rachis length, number of florets per spike, bract length, time of beginning of flowering, corm fresh weight and normal difference vegetative index. Among all quantitative traits, plant height, length of the leaf blade, corm fresh weight, floret length, tepal length and floret width contributed more to the total variation in *Gladiolus* germplasm in the present study. Hybridization among *Gladiolus* genotypes with stable expression and better performance for these traits would be useful in selection of desirable progenies. Variable correlation plot within the circle on bifactorial plane represents contributions of quantitative traits (Figure 6).

Eigen-values demonstrated the PCA's relative discriminating capacity. PCA 1 had the greatest discriminating capacity, as evidenced by its Eigen-value of 3.92, followed by PCA 2 with an Eigen-value of 2.84. Principal component analysis was successful in identifying linear combinations of the 17 different quantitative features that distinguished various *Gladiolus* genotype clusters. A two-dimensional scatter plot of PCA visualized the relationships between *Gladiolus* genotypes (Figure 7). Therefore, PCA revealed that the cumulative variation explained by the first six components accounted over 79.31% variation indicating great variability in quantitative traits, a high genetic diversity between *Gladiolus* genotypes. This suggested that these traits had significant contribution in *Gladiolus* diversity.

Cluster Analysis

Hierarchical cluster analysis based on squared Euclidian distance (distance coefficient=13) classified all 84 *Gladiolus* genotypes in to two major clusters I and II in a dendrogram (Figure 8). Cluster wise mean data of 17 quantitative characteristics is given in Table 5. Cluster I consists of 29 genotypes, which was further classified into two sub clusters Ia and Ib. Clusterwise average data indicated that sub-cluster Ia comprised of 08 genotypes characterized by taller plants (60.08–78.00 cm), longer leaves (47.42–56.67 cm), longer bracts (4.53–6.83 cm), longer florets (9.03–11.17 cm), bigger (5.19–6.88 cm) and heavier corms (44.92–90.83 g) in comparison with other clusters. Sub cluster Ib included 21 genotypes characterized by longest spikes (27–52.25 cm)

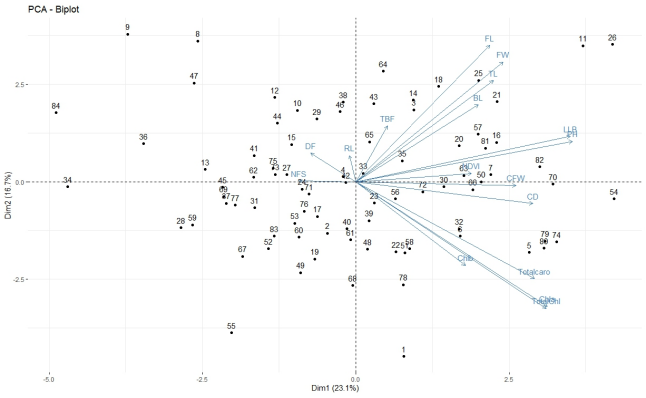

Figure 7: A two-dimensional scatter plot of PCA visualizing the relationships between 84 *Gladiolus* genotypes evaluated during 2017-18 and 2018-19

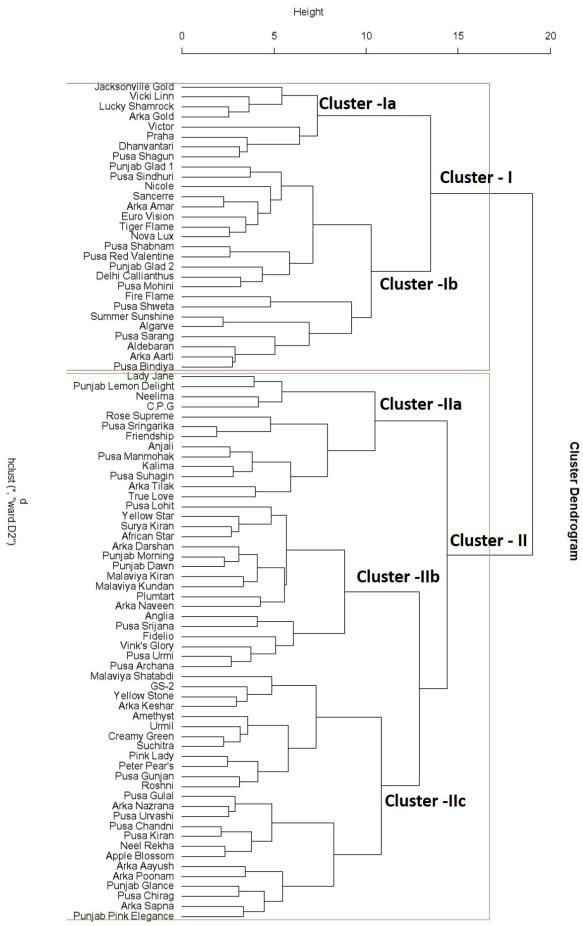

Figure 8: Cluster diagram based on squared Euclidian distance using phenotypic data of 84 *Gladiolus* genotypes evaluated during 2017-18 and 2018-19

with higher floret counts (9.67–17.50), longer tepals (5.72–8.47 cm), rich in chlorophyll and carotenoid contents. The major cluster II consists of 55 genotypes, which was further divided into three sub clusters IIa, IIb and IIc. Sub-cluster IIa consist of 13 genotypes that had broader florets (6.80cm - 11.30 cm) and were late flowering types (102–120 days). Sub-cluster IIb had 17 genotypes characterized by moderate leaf length (35.42–49.83 cm), shorter florets (7.07–9.90 cm) and short flowering duration (7.33–15.83 days). Sub-cluster IIc comprised of 25 genotypes which usually had early flowering genotypes (72.33–114.50 days) with longer flowering duration (7.67–36.50 days). In a similar line of study, Singh *et al*. (2017) morphologically assessed 50 *Gladiolus* cultivars for plant height (80.30–134.70 cm), spike length (40.30–81.70 cm), and number of florets per spike (9.30–18.0). Based on these phenotypic traits, fifty genotypes were grouped into seven primary clusters separated by Euclidean distance coefficients of 14.75. Cluster analysis in our study indicated that genotypes found in sub-cluster Ia are characterized by comparatively taller plants bearing longer florets and heavier corms whereas sub-cluster IIc genotypes possessed early flowering plants with medium spikes with more floret count and longer blooming period. In this way, both clusters are relatively distinct from each other. Hence, genotypes from these clusters can be used in hybridization to obtain desirable progenies. Among all genotypes, cv. Victor is found to be relatively distinct owing to its tallest plants, heavier corms, short flowering duration and maximum corm fresh weight.

Conclusions

This study on 84 genotypes of *Gladiolus* revealed that huge variability exists for 35 morpho-physiological traits in the germplasm sourced from India and abroad. Genotypes for specific traits such as Victor for corm fresh weight and green florets, Malaviya Shatabdi and yellow stone for the longest flowering duration, Praha and Pusa Shagun for plant height and longest leaves, Arka Poonam and Pusa Shabnam for highest count of florets, Vicki Linn, Pusa Sarang and Victor for bigger corms were identified as important material for breeders and for all stake holders. Among all 84 studied genotypes, cv. Victor was found to be relatively distinct owing to its tallest plants, heavier corms, short flowering duration and maximum corm fresh weight. Distinct genotypes identified in the study would help breeders in selecting desirable parents to obtain better segregants. Characterization data generated during the work will assist the breeders in understanding varietal behavior and selection of reliable traits in breeding. It could serve as a reference collection for identifying and cataloguing *Gladiolus* varieties.

Acknowledgments

Authors acknowledge ICAR-IARI, New Delhi for providing Senior Research Fellowship to the first author.

References

Arnon, D. I. (1949). Copper enzymes in intact chloroplast. Polyphenoxidase in Beta vulgaris. *Plant Physiology*, *24*, 1-15.

Cantor, M., & Tolety, J. (2011). Gladiolus. In Wild Crop Relatives: *Genomic and Breeding Resources*: *Plantation and Ornamental Crops* (pp. 133-159). Berlin, Heidelberg: Springer Berlin Heidelberg.

Choudhary, M., Moond, S. K., & Kumari, A. (2011). Correlation studies in gladiolus. *Res. Plant Biol.*, **1(4):** 68-72.

Datta, S. K., & Chakrabarty, D. (2016). Classical and modern methods for the characterization of ornamental crops. *Int. J. Hortic.*, **5(21):** 1-45.

De Vicente, M. C., Guzmán, F. A., Engels, J., & Rao, V. A. (2006). Genetic characterization and its use in decision-making for the conservation of crop germplasm. In J. Ruane & A. Sonnino (Eds.), *The Role of Biotechnology in Exploring and Protecting Agricultural Genetic Resources* (pp. 129-138). FAO of the United Nations, Rome.

Goldblatt, P., & Manning, J. C. (2008). *The Iris family: natural history & classification*. Timber Press.

Goldblatt, P., Manning, J. C., Von Blittersdorff, R., & Weber, O. (2014). New species of *Gladiolus* L. and Moraea Mill. (Iridaceae) from

Tanzania and Mozambique. *Kew Bulletin*, *69*(2), 1-8.

Hiscox, J. D., & Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canad. J. Bot.*, **57(12):** 1332-1334.

Hort, D. A., Cantor, M., & Erzsebet, B. (2014). Heritability of quantitative traits with an important role for improving Gladiolus hybridus assortment. *Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Horticulture*, **71(1):** 50-55.

Imanishi, H. (1989). Gladiolus. In T. Matsuo (Ed.), Collected data of plant genetic resources (pp. 1077-1080). *Kodansha Scientific*, Tokyo.

Kadam, G. B., Kumar, G., Saha, T. N., Tiwari, A. K., & Kumar, R. (2014). Varietal evaluation and genetic variability studies on Gladiolus. *Indian J. Hortic.*, **71(3):** 379-384.

Kalloo, G., Srivastava, U., Nalavadi, U. G., Mishra, R. L., Kumar, S., Goel, A. K., & Singh, T. (2013). Guidelines for the conduct of test for distinctiveness, uniformity, and stability on Gladiolus (*Gladiolus spp.* L.). *Protection of Plant Varieties and Farmers' Rights Authority* (PPV & FRA), Government of India.

Kumar, P. H., & Kulkarni, B. S. (2009). Genetic variability in Gladiolus for growth and flowering characters (*Gladiolus* × *hybridus* Hort.). *J. Hortic. Sci.*, **4(2):** 177-180.

Lichtenthaler, H. K., & Wellburn, W. R. (1983). Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.*, **11:** 591-592.

Pattanaik, S., Paul, A., & Lenka, P. C. (2015). Genotypic and phenotypic variability and correlation studies in Gladiolus. *J. crop weed.*, **1(1):** 113-119.

Ramzan, A., Nawab, N. N., Ahad, A., Hafiz, I. A., Tariq, M. S., & Ikram, S. (2016). Genetic variability, correlation studies, and path coefficient analysis in *Gladiolus alatus* cultivars. *Pak. J. Bot.*, **48(4):** 1573-1578.

Singh, N., Pal, A. K., Meena, B., Roy, R. K., Tamta, S., & Rana, T. S. (2017). Development of ISSR and RAPD-derived SCAR markers for identification of Gladiolus germplasm. *J. Hortic. Sci. Biotechnol.*, **92(6):** 577-582.

Singh, N., Pal, A. K., Roy, R. K., Tewari, S. K., Tamta, S., & Rana, T. S. (2018). Characterization of Gladiolus germplasm using morphological, physiological, and molecular markers. *Biochem. Genet.*, **56(1-2):** 128-148.

Sinha, P., & Roy, S. K. (2002). Plant regeneration through in vitro cormel formation from callus culture of *Gladiolus primulinus* Baker. *Plant Tissue Cult.*, **12(2):** 139-145.

Szczepaniak, M., Kaminski, R., Kuta, E., Słomka, A., Heise, W., & Cieslak, E. (2016). Natural hybridization between *Gladiolus palustris* and *Gladiolus imbricatus* inferred from morphological, molecular, and reproductive evidence. *Preslia*, **88(1):** 137-161.

Zaharia, A., Cantor, M., Borsai, O., Erzsebet, B., & Jucan, D. (2018). Correlation analysis of different morphological characters among cultivars and hybrids of *Gladiolus hybridus* Hort. *Rom. Biotechnol. Lett.*, **12(2):** 13723-13727.

Zahoor, A., Dhatt, K. K., Ganai, N. A., Dar, Q. A. H., & Nageena, N. (2019). Analysis of genetic diversity in Gladiolus (*Gladiolus hybridus*) by multivariate analysis under sub-tropical conditions of Punjab (India). *Int. J. Agric. Sci.*, **15(1):** 167-172.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2022.00036.2.10

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Exploring the Hidden Potential of Traditional Rice Landraces and Mutants for Iron and Zinc Concentration in Brown Rice to Develop Bio-fortified Rice Varieties

Deepika Parte[1], Parmeshwar K. Sahu[1], Ravi Raj S. Patel[1], Richa Sao[1], Samrath Baghel[1], Kuber Bhainsa[2], B.K. Das[2] and Deepak Sharma[1*]

Abstract

In the present study, 58 rice genotypes were evaluated for three consecutive seasons to enucleate the elite lines with high Fe and Zn contents. ANOVA revealed significant differences among the genotypes for Fe and Zn contents during all three seasons. Furthermore, PCV and GCV were moderate to high for Fe and Zn content during all three seasons. Similarly, high level of heritability and genetic advance were also recorded for both the traits. Iron content of Safri mutant 17-2-48 was consistently high for all the seasons whereas zinc content of Loktimachi was high for two seasons. Interestingly, rice genotypes Chhindmauri, Badsahbhog, Rudra, Danighoda and Suapankhi have been identified to have moderate to high Fe and Zn contents which could be used as donors for developing bio-fortified rice varieties. Stability analysis revealed that the genotypes Jhimiprass, Loktimachi, and CG Zinc Rice 2 for iron content and Rela Dhan, Chhindmauri, and CG Zinc Rice-1 for zinc content have had an average response to environment, making them appropriate for all seasons

Keywords: Rice landraces, Bio-fortification, Fe & Zn content, Variability, Stability analysis.

[1]Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India.

[2]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre (BARC), Mumbai, Maharashtra, India.

***Author for correspondence:**

deepak1962@igkv.ac.in

Received: 20/09/2022 **Revised:** 20/02/2023

Accepted: 22/05/2023

How to cite this article: Parte, D., Sahu, P.K., Patel, R.R.S., Sao, R., Baghel, S., Bhainsa, K., Das, B.K., Sharma, D. (2023). Exploring the Hidden Potential of Traditional Rice Landraces and Mutants for Iron and Zinc Concentration in Brown Rice to Develop Bio-fortified Rice Varieties. *Indian J. Plant Genetic Resources*. 36(2), 280-289. **DOI:** 10.5958/0976-1926.2022.00036.2.10

Introduction

Rice is a major staple food for more than half of the world's population and contributes to solve the problem of hunger among poor people, but they are rarely accessible to nutrient rich food sources. The prevalence of micronutrient deficiency, especially for crucial elements like Fe content and Zn content among the rice-consuming population, prompted in initiation bio-fortification programme in rice which has been identified as a sustainable source to develop nutrient-rich rice grains to poor people who depend solely on rice for the energy and nutrients (Rao *et al*., 2014). Traditional rice landraces have tremendous genetic variability and possess valuable genes for nutritional traits. Several biotic and abiotic stresses have adaptability in wide agroecological niches, unmatched qualitative traits and therapeutic properties, and thus possesses great genetic potential for rice improvement. Along with the rice landraces, mutants developed from them are a valuable source of micronutrients that need to be quantified and utilized to develop bio-fortified rice varieties. Production of varieties containing high amounts of bioavailable Fe would improve Fe nutrition in regions with prevalent iron deficiency (Aggett, 2020; Maret and Sandstead, 2006; Shahzad *et al*., 2014). Studies by Harvest Plus and others have shown considerable iron and zinc

losses during rice polishing. Therefore, an increase in the concentration of Fe and Zn in brown rice is a high-priority research area and later on that could be quantified in white rice for varietal development (Ryu and Aydemir, 2020). For this concern, we need to focus on micronutrient-rich sources which can be easily reachable and acceptable to people.

Biofortification is one of the sustainable approaches for improving the iron and zinc content and their bioavailability in rice grains. Nevertheless, before the implementation of thebiofortification process, the primary step in conventional breeding is to screen out the micronutrient-dense cultivars within the natural existing germplasm because the presence of awide range of genetic variations in the population helps in selecting the desired genotypes. Furthermore, selecting stable genotypes for high micronutrient content is also crucial for developing nutri-rich rice varieties. Therefore, the current study utilized 44 rice landraces and 8 mutants along with the 6 check to explore the genetic variability, stability and hidden potential of traditional rice landraces and mutants for iron and zinc concentration in brown rice over different seasons. Moreover, the outcome of this study will be further helpful to create the genetic database for breeding program specific for improving the micronutrient in the rice varieties.

Materials and Methods

Experimental Site

The field experiments were conducted at Instructional Farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492012 (C.G.) India, during three seasons *viz*., *Kharif* season-2020, *Rabi* season -2020-21, and *Kharif* season -2021. Geographically, Chhattisgarh state of India is located between 17°14′ N and 24°45′ N latitude and longitudeof 79°16′ E. The experiment was laid out by following a randomized complete block design with two replications during all three seasons. All the standard agronomic practices were adopted during the crop seasons.

Plant Materials

A total of 58 accessions, including 44 landraces, 08 mutants, and 06 checks, were used in the experiment. All the genotypes were accessed from the Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492012 (C.G.). The list of experimental materials is presented in Table 1. Dehusked brown rice was used to estimate Zinc and Iron content during all three seasons.

Estimation of Iron and Zinc Contents

Iron and zinc content were estimated using the S2 RANGER ED-XRF Method at International Rice Research Institute, South Asia Hub, ICRISAT Campus, Hyderabad, India center. The seed samples were dehusked by the help of Zaccaria testing rice mill having rubber roller. Before dehusking, the grains were washed with 0.1 N HCl followed by rinsing with double distilled water to make the sample free from contamination. Total 5 g of properly cleaned brown rice in triplicates were used for the estimation of Fe and Zn contents. The published protocol of ED-XRF was used to estimate both the micro-nutrients (Rao *et al.,* 2014). The data from the application was exported in MS Excel sheet for further analysis.

Table 1: List of rice genotypes used as experimental materials under the study

S. No.	*Name of genotypes*	*Parentage*
1	Red Barhasal	landrace
2	Rudra	landrace
3	Tulsibhog	landrace
4	Loktimachi	landrace
5	Odha Dhan	landrace
6	Gathuwan Dhan	landrace
7	Raja Bangla	landrace
8	Brown Rice-1	landrace
9	Munibhog	landrace
10	Danighoda	landrace
11	Saraitolia	landrace
12	Pangudi Goindi	landrace
13	Safri-17	landrace
14	Dubraj	landrace
15	Byalo	landrace
16	Badshahbhog	landrace
17	Jonyaphool	landrace
18	Mohlyanbanko	landrace
19	Kanakbhog	landrace
20	Shwet Ganga	landrace
21	Matko Dhan	landrace
22	Suapankhi	landrace
23	Jhimiprass	landrace
24	Jauphool	landrace
25	Kalajeera	landrace
26	Chhindmauri	landrace
27	Motipeera	landrace
28	Pedgadi	landrace
29	Sonagathi	landrace
30	Jana Dhan	landrace
31	Rela Dhan	landrace
32	Ram Shree	landrace
33	Kari Alcha	landrace
34	Jhilli Safri	landrace
35	Siyar	landrace
36	Karhani	landrace

37	Nandel	landrace
38	Safri	landrace
39	Dhaura Mundariya	landrace
40	Karhani-2	landrace
41	Sanchuriya	landrace
42	Alsakar	landrace
43	Luchai	landrace
44	Mai Dubraj	landrace
45	TCDM-1	Mai Dubraj
46	Vikram-TCR	Safri-17
47	C.G. Jawaphool Trombay	Jawaphool
48	Alsakar Mutant-3	Alsakar
49	Sanchuriya Mutant-6	Sanchuriya
50	Safri-17-5 Mutant	Safri-17
51	Safri-17-2-48 Mutant	Safri-17
52	Luchai Mutant -1	Luchai
53	Dubraj Sel-1	Dubraj
54	CG Zinc Rice 1	Poornima x Annada
55	CG Zinc Rice 2	IR681444-B-18-2-1-1 x HMT
56	Zinco Rice MS	Lalmati x IR681444
57	Rajeshwari	R320-300 x ChaptiGurmatiya
58	Protazin	Ciherang x R-7586-106-28-3-B-B-B

Statistical Analysis

Statistical analysis for analysis of variance (ANOVA), genetic variability parameters like mean, range, phenotypic coefficients of variation (PCV), genotypic coefficients of variation (GCV), heritability in broad sense (Hb), and genetic advance as percent of the mean parameters were calculated through the standard software (Windostat version 9.2 from Indostat services, by following the standard methodology. Stability analysis over the three seasons was performed following the Eberhart and Russell (1966) Model through Windostat version 9.2 from Indostat services.

Results

Total 58 accessions were analyzed and the result of pooled variance analysis (Table 2) revealed high variability for the iron and zinc as represented by the highly significant mean sum of squares over three seasons. However, the results of different genetic variability parameters revealed that the phenotypic coefficients of variation (PCV) were slightly higher than the corresponding genotypic coefficients of variation (GCV) for both traits among all three seasons (Table 3). Heritability in broad sense (Hb) were found to be medium for iron (36.30%) and zinc (41.78%) in season I while in season II it was estimated high for Iron (83.54%) and Zinc (80.21%). In season III heritability was high for Zn (73.17%) but found medium for Fe (64.68%). Similarly, high estimate of genetic advance as a percent of mean was recorded for Fe in season II (39.92) and season III (27.3), while it was estimated as medium in season I (16.66). However, genetic advance as a percent of mean for Zn was estimated to be medium in all three seasons (Table 3). Total 58 accessions were analyzed and variation in iron and zinc contents in brown rice was studied in different seasons.

Table 2: Pooled analysis of variance (ANOVA) for Fe and Zn contents over the three seasons (*Kharif season* 2020, Rabi season 2020-21, and *Kharif season* 2021.)

Source of variation	*Degree of freedom*	*Mean sum of square*	
		Fe	*Zn*
Replications	1	5.945	4.661
Genotypes	57	28.017**	36.577**
Environment	2	5.413	6.977
Genotype x environment	2	12.916**	23.267**
Errors	285	2.273	2.765

** Significant at 1% level of significance, * Significant at 5% level of significance

In season I, range for the concentration of iron varied from 8.13 ± 0.46 to 20.83 ± 0.71 ppm with an average of 11.41 ppm iron, while zinc content among the 58 genotypes ranged from 14.41 ± 1.29 to 27.45 ± 1.45 ppm with an average of 21.41 ppm zinc. Among all genotypes, Safri mutant 17-2-48 had the highest iron content (20.83 ppm), while Red Barhasal recorded the lowest iron content during *Kharif*-2020.

Meanwhile, Munibhog recorded the highest zinc content and Odha Dhan recorded the least (Table 4). Among the genotype tested, 78% were found high for iron content while 22% of genotypes had low iron content, 74% contained high zinc, and 26% had low zinc content (Figure 1) in season I. The top five elite genotypes with high iron during season I are Safri-17-2-48 Mutant, Badshahbhog, Rajeshwari, Chhindmauri, and Rudra (Table 5). The top five elite

Table 3: Genetic variability parameters for Fe and Zn contents over the three seasons

Micronutrients	*Kharif* season 2020					*Rabi* season 2020-21					*Kharif* season 2021				
	Mean	PCV %	GCV %	Hb	GA % mean	Mean	PCV %	GCV %	Hb	GA % mean	Mean	PCV %	GCV %	Hb	GA % mean
Fe	11.42	22.29	13.43	36.30	16.66	11.85	23.2	21.21	83.54	39.92	11.63	20.47	16.46	64.68	27.3
Zn	21.42	15.69	10.14	41.78	13.51	20.93	12.47	11.17	80.21	20.61	21.18	12.58	10.76	73.17	19

genotypes with high zinc are Munibhog, Byalo, Jhimiprass, Jonyaphool, and Loktimachi (Table 6). Iron concentration in season II ranged from 8.31 ± 0.01 ppm to 22.19 ± 2.63 ppm and zinc concentration from 16.33 ± 1.03 to 25.80 ± 0.01 ppm with the mean value of 11.85 and 20.93 ppm for iron and zinc respectively (Table 4). Meanwhile, out of tested genotypes, 83% of total genotypes had high iron content while 17% of genotypes have low iron content, whereas for a concentration of zinc 67% of genotypes contained high zinc while 33% has low zinc in brown rice (Figure 2).

The top elite genotypes with high iron during season II are Safri-17-2-48 Mutant, TCDM-1, Rudra, Chhindmauri, and Suapankhi, respectively (Table 5). The top elite genotypes with high zinc are Loktimachi, TCDM-1, Jhimiprass, Badshahbhog, and Chhindmauri (Table 6). Chhindmauri and TCDM-1 can be used as the donor parent for both iron and zinc.

However, variation in iron during the third season ranged from 8.68 ± 0.35 (Raja Bangla) to 21.51 ± 0.96 ppm (Safri-17-2-48 Mutant) and zinc concentration from 15.38 ± 1.37 (Odha Dhan) to 25.80 ± 0.01 ppm (Loktimachi) with the mean value of 11.63 ppm for iron and 21.18 ppm for zinc (Table 4). Out of 58 genotypes, 84% found high iron while 16% have low iron content, 67% contain high zinc while 33% have low zinc, as shown in Figure 3. The top five genotypes with high iron during season III are Safri 17-2-48 Mutant, Rudra, Chhindmauri, TCDM-1, and Badshahbhog (Table 5). While the top five genotypes which having high zinc are Loktimachi, Byalo, Munibhog, Jhimiprass, and Chhindmauri (Table 6) these genotypes can be utilized to develop biofortified varieties.

Among all the seasons, the concentration of Iron was found high for Safri mutant 17–2-48, where Munibhog recorded for highest zinc concentration during season I, whereas Loktimachi found the highest during the second and third seasons. Thus, variations in rice grains' iron and zinc content were observed during different seasons in the present study. These variations can be attributed to different factors such as environment, soil properties, nature of grain, genotype, and interaction of genotype X environment, micronutrient homeostasis, method of analysis, *etc*. Furthermore, Chhindmauri, Badshahbhog, Rudra, Danighoda, Suapankhi, TCDM-1 and Luchai Mutant-1 exhibited best for both Zn and Iron. Thus, these genotypes can be used as donor parents for developing Iron and Zinc rich varieties.

To test the significance among the genotypes, mean sum of square due to genotypes has been tested against mean sum of square due to pooled deviation. The results indicated highly significant mean sum of square due to genotypes for both iron and zinc contents in brown rice, indicating the availability of ample variability among the genotypes (Table 7). Stability analysis revealed that all the genotypes included in the study deviated non-significantly from zero

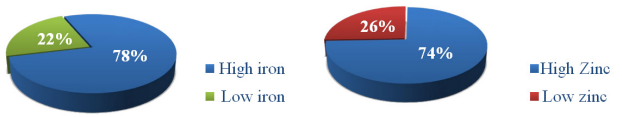

Figure 1: Percentage of genotypes having high and low Iron and Zinc content during *Kharif* season 2020 (season I)

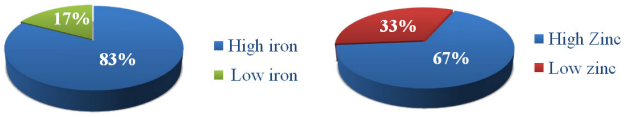

Figure 2: Percentage of genotypes having high and low Iron and Zinc content during *Rabi* season 2020-21 (season II)

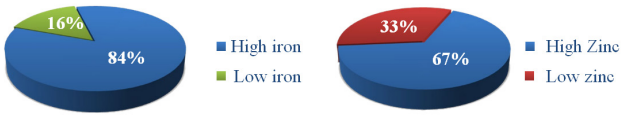

Figure 3: Percentage of genotypes having high and low Iron and Zinc content during *Kharif* season 2021 (season III)

($\sigma^2d=0$). Still, only genotypes Jhimiprass, Loktimachi and CG zinc rice two deviated non-significantly from unity of regression for iron content. The 3 genotypes *viz*., Rela Dhan, Chhindmauri and CG Zinc Rice 1 were deviated non-significantly from unity of regression for zinc content (Table 8). Hence these genotypes have an average response to environments and are recommended for all seasons (environments), while the regression coefficient of 32 genotypes for iron and 27 genotypes for zinc recorded significant deviation from unity and less than unity ($b<1$). Hence these genotypes have below average response to environments and are recommended for poor unfavorable environments. Furthermore, the regression coefficient of 23 genotypes for iron and 25 genotypes for zinc recorded significant deviation from unity and more than unity ($b> 1$). Therefore, it has an above-average response to environments and is recommended for favorable environments.

Discussion

Iron and zinc deficiency is notably the most widespread micronutrient deficiency among the human population. As a staple food, a lot of efforts are being made to enrich the nutritional status of rice to prevent malnutrition. However, the effect of these efforts is still limited because of the low consumer acceptability of fortified food and the low bioavailability of iron and zinc in our resources (Pyo *et al.,* 2022). For this concern, knowledge of our germplasm resources and their preferentially elemental constitutions of the different grain tissues and their remobilization patterns during germination makes the possibility of designing target products with a nutritionally optimal constitution more feasible (Arora *et al*., 2010; Omary *et al*., 2012). While a better understanding of the physiological basis of nutrient

Table 4: Mean performance of rice landraces and mutant for Fe and Zn contents over the three seasons

Treatments (Genotypes)	*Season 1*		*Season 2*		*Season 3*	
	Fe (ppm)	*Zn (ppm)*	*Fe (ppm)*	*Zn (ppm)*	*Fe (ppm)*	*Zn (ppm)*
Red Barhasal	8.13 ± 0.46	16.45 ± 2.76	10.95 ± 1.48	21.03 ± 3.08	9.54 ± 2.00	18.74 ± 3.24
Rudra	14.55 ± 3.61	23.85 ± 2.33	18.45 ± 3.04	20.03 ± 1.03	16.50 ± 2.76	21.94 ± 2.70
Tulsibhog	13.00 ± 2.12	22.33 ± 0.04	12.00 ± 0.71	22.30 ± 0.01	12.50 ± 0.71	22.31 ± 0.02
Loktimachi	11.80 ± 0.57	25.80 ± 0.02	12.20 ± 0.01	25.80 ± 0.01	12.00 ± 0.28	25.80 ± 0.00
Odha Dhan	9.78 ± 2.65	14.41 ± 1.29	13.00 ± 2.40	16.35 ± 1.34	11.39 ± 2.28	15.38 ± 1.37
Gathuwan Dhan	11.30 ± 5.52	22.13 ± 3.57	10.85 ± 1.63	23.13 ± 1.66	11.08 ± 0.32	22.63 ± 0.71
Raja Bangla	8.93 ± 2.30	24.03 ± 5.83	8.43 ± 1.60	19.90 ± 0.00	8.68 ± 0.35	21.96 ± 2.92
Brown Rice-1	11.58 ± 0.81	21.63 ± 1.59	11.90 ± 0.42	22.15 ± 0.78	11.74 ± 0.23	21.89 ± 0.37
Munibhog	11.20 ± 1.56	27.45 ± 1.45	10.10 ± 0.00	23.70 ± 0.01	10.65 ± 0.78	25.58 ± 2.65
Danighoda	11.23 ± 0.46	23.53 ± 0.92	13.00 ± 0.99	22.48 ± 0.04	12.11 ± 1.26	23.00 ± 0.74
Saraitolia	9.53 ± 0.67	22.68 ± 1.45	10.75 ± 0.35	22.35 ± 0.64	10.14 ± 0.87	22.51 ± 0.23
Pangudi Goindi	9.10 ± 19.8	22.95 ± 0.92	10.50 ± 0.01	22.30 ± 0.00	9.80 ± 0.99	22.63 ± 0.46
Safri-17	10.00 ± 2.12	21.65 ± 5.3	11.50 ± 0.00	17.90 ± 0.01	10.75 ± 1.06	19.78 ± 2.65
Dubraj	10.08 ± 0.88	22.53 ± 1.66	11.15 ± 0.21	20.18 ± 1.66	10.61 ± 0.76	21.35 ± 1.66
Byalo	12.23 ± 1.73	27.15 ± 4.31	11.00 ± 0.00	24.10 ± 0.00	11.61 ± 0.87	25.63 ± 2.16
Badshahbhog	16.55 ± 2.76	23.28 ± 1.94	15.43 ± 0.39	24.68 ± 1.31	15.99 ± 0.80	23.98 ± 0.99
Jonyaphool	10.63 ± 1.66	25.98 ± 1.94	10.23 ± 0.74	21.45 ± 1.48	10.43 ± 0.28	23.71 ± 3.20
Mohlyanbanko	11.45 ± 0.64	18.42 ± 2.57	12.43 ± 0.25	20.58 ± 1.87	11.94 ± 0.69	19.50 ± 1.53
Kanakbhog	9.40 ± 1.13	16.77 ± 0.90	8.76 ± 0.52	18.50 ± 0.19	9.08 ± 0.45	17.63 ± 1.22
Shwet Ganga	10.83 ± 0.75	22.66 ± 2.04	10.38 ± 0.04	21.03 ± 1.45	10.60 ± 0.32	21.84 ± 1.16
Matko Dhan	12.55 ± 1.06	23.88 ± 2.3	11.13 ± 1.03	18.53 ± 3.29	11.84 ± 1.01	21.20 ± 3.78
Suapankhi	9.28 ± 1.24	21.48 ± 2.93	16.50 ± 3.82	20.30 ± 0.42	12.89 ± 5.11	20.89 ± 0.83
Jhimiprass	11.03 ± 0.67	26.23 ± 1.87	11.50 ± 0.00	24.90 ± 0.01	11.26 ± 0.34	25.56 ± 0.94
Jauphool	11.30 ± 0.14	20.80 ± 5.09	11.20 ± 0.00	17.20 ± 0.01	11.25 ± 0.07	19.00 ± 2.55
Kalajeera	11.92 ± 0.59	22.92 ± 1.11	11.50 ± 0.01	23.70 ± 0.00	11.71 ± 0.29	23.31 ± 0.56
Chhindmauri	14.83 ± 4.35	25.10 ± 0.71	17.90 ± 0.00	24.60 ± 0.00	16.36 ± 2.17	24.85 ± 0.35
Motipeera	10.40 ± 0.85	22.12 ± 1.11	10.10 ± 0.14	22.83 ± 0.04	10.25 ± 0.21	22.47 ± 0.50
Pedgadi	11.72 ± 1.01	24.16 ± 3.05	11.00 ± 0.00	23.00 ± 1.41	11.36 ± 0.51	23.58 ± 0.82
Sonagathi	9.80 ± 0.01	19.30 ± 3.25	10.85 ± 0.49	17.83 ± 0.39	10.33 ± 0.74	18.56 ± 1.04
Jana Dhan	11.68 ± 4.35	21.23 ± 10.2	10.18 ± 0.74	17.08 ± 1.45	10.93 ± 1.06	19.15 ± 2.93
Rela Dhan	10.20 ± 2.97	21.56 ± 5.32	9.72 ± 0.69	20.98 ± 3.32	9.96 ± 0.34	21.27 ± 0.41
Ram Shree	10.11 ± 2.84	17.41 ± 1.54	11.85 ± 1.77	16.33 ± 1.03	10.98 ± 1.23	16.87 ± 0.77
Kari Alcha	9.27 ± 1.36	21.68 ± 0.45	9.88 ± 0.74	23.50 ± 0.71	9.57 ± 0.43	22.59 ± 1.29
Jhilli Safri	11.55 ± 0.35	22.63 ± 2.86	11.58 ± 0.11	20.60 ± 0.00	11.56 ± 0.02	21.61 ± 1.43
Siyar	12.77 ± 0.65	22.28 ± 2.75	14.95 ± 0.54	21.18 ± 2.75	13.86 ± 1.54	21.73 ± 0.78
Karhani	12.92 ± 0.83	22.11 ± 2.81	9.90 ± 0.97	22.50 ± 0.71	11.41 ± 2.14	22.31 ± 0.28
Nandel	10.60 ± 2.97	21.75 ± 1.2	12.25 ± 1.77	22.03 ± 0.53	11.43 ± 1.17	21.89 ± 0.19
Safri	11.18 ± 1.45	22.60 ± 1.27	10.28 ± 1.73	21.73 ± 0.56	10.73 ± 0.63	22.16 ± 0.62
Dhaura Mundariya	9.64 ± 1.61	15.00 ± 1.41	9.25 ± 0.35	16.55 ± 1.2	9.45 ± 0.28	15.78 ± 1.10
Karhani-2	10.26 ± 0.04	20.69 ± 0.53	9.87 ± 0.90	21.47 ± 0.19	10.06 ± 0.28	21.0 ± 0.55
Sanchuriya	9.01 ± 1.40	18.00 ± 1.98	10.75 ± 0.35	18.05 ± 0.64	9.88 ± 1.23	18.03 ± 0.04
Alsakar	10.74 ± 1.90	20.71 ± 0.69	12.63 ± 1.52	21.95 ± 0.35	11.68 ± 1.33	21.33 ± 0.88
Luchai	11.30 ± 1.27	20.83 ± 1.38	11.06 ± 0.23	21.16 ± 1.34	11.18 ± 0.17	20.99 ± 0.23

Mai Dubraj	9.48 ± 0.11	21.65 ± 0.21	12.03 ± 1.24	22.18 ± 0.18	10.75 ± 1.80	21.91 ± 0.37
TCDM-1	13.00 ± 2.83	22.50 ± 0.71	19.48 ± 1.20	24.99 ± 0.48	16.24 ± 4.58	24.62 ± 0.52
Vikram-TCR	11.50 ± 0.71	18.35 ± 0.49	11.30 ± 0.14	18.33 ± 0.18	11.40 ± 0.14	18.34 ± 0.02
CG Jawaphool Trombay	10.96 ± 1.05	20.45 ± 0.64	9.68 ± 0.95	18.95 ± 0.92	10.32 ± 0.91	19.70 ± 1.06
Alsakar Mutant-3	11.07 ± 0.23	17.06 ± 1.20	12.18 ± 0.60	16.55 ± 0.64	11.62 ± 0.78	16.80 ± 0.36
Sanchuriya Mutant-6	11.32 ± 2.71	17.77 ± 2.03	13.53 ± 1.94	16.95 ± 1.06	12.42 ± 1.56	17.36 ± 0.58
Safri-17-5 Mutant	11.20 ± 1.13	17.86 ± 0.48	10.40 ± 0.00	18.95 ± 0.35	10.80 ± 0.57	18.41 ± 0.77
Safri-17-2-48 Mutant	20.83 ± 0.71	17.82 ± 0.26	22.19 ± 2.63	19.25 ± 0.10	21.51 ± 0.96	18.53 ± 1.01
Luchai Mutant-1	13.87 ± 0.52	21.82 ± 0.69	11.55 ± 0.92	21.85 ± 0.21	12.71 ± 1.64	21.83 ± 0.02
Dubraj Sel-1	10.67 ± 2.35	21.02 ± 0.45	10.50 ± 0.71	23.25 ± 1.20	10.58 ± 0.12	22.13 ± 1.58
CG Zinc Rice-1	9.70 ± 0.71	22.34 ± 1.33	8.31 ± 0.01	21.84 ± 1.19	9.01 ± 0.98	22.09 ± 0.35
CG Zinc Rice-2	12.50 ± 0.71	22.50 ± 0.71	13.00 ± 1.41	23.50 ± 0.71	12.50 ± 0.71	22.50 ± 0.71
Zinco Rice MS	12.07 ± 0.37	19.93 ± 1.80	9.93 ± 0.88	18.80 ± 1.13	11.00 ± 1.51	19.36 ± 0.80
Rajeshwari	16.03 ± 7.46	22.20 ± 0.57	11.49 ± 1.19	20.82 ± 0.73	13.76 ± 3.21	21.51 ± 0.98
Protazin	12.65 ± 0.35	18.81 ± 0.69	12.81 ± 0.57	19.61 ± 0.40	12.73 ± 0.11	19.21 ± 0.57

Table 5: Top performing genotypes for Fe contents in rice genotypes

	Season 1 (*Kharif* season 2020)		Season 2 (*Rabi* season 2020-21)		Season 3 (*Kharif* season 2021)	
	Genotypes	Fe (ppm) content in brown rice	Genotypes	Fe (ppm) content in brown rice	Genotypes	Fe (ppm) content in brown rice
1	Safri-17-2-48 Mutant	20.83 ± 0.71	Safri-17-2-48 Mutant	22.19 ± 2.63	Safri-17-2-48 Mutant	21.51 ± 0.96
2	Badshahbhog	16.55 ± 2.76	TCDM-1	19.48 ± 1.20	Rudra	16.50 ± 2.76
3	Rajeshwari	16.03 ± 7.46	Rudra	18.45 ± 3.04	Chhindmauri	16.36 ± 2.17
4	Chhindmauri	14.83 ± 4.35	Chhindmauri	17.90 ± 0.00	TCDM-1	16.24 ± 4.58
5	Rudra	14.55 ± 3.61	Suapankhi	16.50 ± 3.82	Badshahbhog	15.99 ± 0.80

Table 6: Top performing genotypes for Zn contents in rice genotypes

	Season 1		Season 2		Season 3	
	Genotypes	Zn (ppm)content in brown rice	Genotypes	Zn (ppm)content in brown rice	Genotypes	Zn (ppm)content in brown rice
1	Munibhog	27.45 ± 1.45	Loktimachi	25.80 ± 0.01	Loktimachi	25.80 ± 0.01
2	Byalo	27.15 ± 4.31	TCDM-1	24.99 ± 0.48	Byalo	25.63 ± 2.16
3	Jhimiprass	26.23 ± 1.87	Jhimiprass	24.90 ± 0.01	Munibhog	25.58 ± 2.65
4	Jonyaphool	25.98 ± 1.94	Badshahbhog	24.68 ± 1.31	Jhimiprass	25.56 ± 0.94
5	Loktimachi	25.80 ± 0.02	Chhindmauri	24.60 ± 0.00	Chhindmauri	24.85 ± 0.35

uptake (Kong *et al*., 2022), their translocation, and the maintenance of homeostasis in grain is essential for the genetic biofortification of rice, complete knowledge of these processes in rice is still lacking (Olsen and Palmgren, 2014; Kobayashi *et al.,* 2021).

Ultimately, the concentration of micronutrients in rice grain depends on the genotype's efficiency to uptake and translocate the nutrients from root to the grain, which involves many complex physiological processes at different levels. Therefore, rice genotypes to be developed should have the genetic potential and physiological efficiency to utilize the available micronutrients from the soil (Olsen and Palmgren, 2014). Knowledge of mineral localization within rice grains is important for understanding the role of different elements in seed development and facilitating the biofortification of seed micronutrients to enhance seeds' values in human diets (Lu *et al*., 2013).

To initiate a breeding program to improve or develop any variety depends on the availability of variation in germplasm for the targeted trait, which can be further used in genetic studies, to make crosses, molecular marker development, and to understand the basis of the uptake process for micronutrients. A large genetic variation for grain iron and zinc has been observed in different germplasm of rice and it was exploited in breeding programs. In our study Iron content in season I, ranged between 8.13 ± 0.46 to

Table 7: Analysis of variance for stability of iron and zinc content among rice genotypes

Source of variation	*Mean sum of squares*		
	Degree of Freedom	*Fe (ppm)*	*Zn (ppm)*
Replications within Environment	3	5.297 **	8.525 **
Genotypes	57	14.009 **	18.289 **
Env.+ (Gen.* Env.)	116	1.029 **	1.043 **
Environments	2	2.706 **	3.488 **
Gen.* Env.	114	0.999 **	1.000 **
Environments (Lin.)	1	5.412 **	6.977 **
Gen.* Env. (Lin.)	57	1.997 **	1.987 **
Pooled Deviation	58	0.001	0.013
Pooled Error	171	1.228	1.638
Total	173	5.305	6.725

20.83 ± 0.71 ppm and zinc content between 14.41 ± 1.29 to 27.45 ± 1.45 ppm in brown rice. Similarly, in season II, Iron content ranged from 8.31 ± 0.01 to 22.19 ± 2.63 ppm and zinc from 16.33 ± 1.03 to 25.80 ± 0.01 ppm. Interestingly, the range of micronutrients were slightly higher in season III, *viz*., 8.68 ± 0.35 to 21.51 ± 0.96 ppm for iron content and 15.38 ± 1.37 to 25.80 ± 0.01 ppm for zinc content. Zhang *et al.,* (2018) experimented on a set of 698 rice germplasms and found that the iron concentration ranged between 0.9 to 9.1 µg/g while zinc concentration ranged from 5.8 to 29.6 µg/g in the polished rice. Similarly, Roy and Sharma (2014) found ample variation for Fe and Zn content in 84 rice cultivars and reported that Iron content varied between 0.25 to 34.8 µg/g and zinc content from 0.85 to 195.3 µg/g where local cultivars had the highest iron content. The grain iron content was found to be relatively higher in the second season with the maximum values of 22.19 ± 2.63

Table 8: Mean performance and stability parameters for Iron content (ppm) and Zinc content (ppm) of rice genotypes

Genotypes	Fe (ppm)			Zn (ppm)		
	Mean (µ)	βi	σ^2di	Mean (µ)	βi	σ^2di
Red Barhasal	9.54	6.54**	-1.30 ^{NS}	18.74	-9.33**	-1.75 ^{NS}
Rudra	16.50	9.03**	-1.30 ^{NS}	21.94	7.80*	-1.76 ^{NS}
Tulsibhog	12.50	-2.31**	-1.30 ^{NS}	22.31	0.05**	-1.76 ^{NS}
Lokti musi	12.00	0.93 ^{NS}	-1.30 ^{NS}	25.80	0.00**	-1.76 ^{NS}
Odha dhan	11.39	7.46**	-1.30 ^{NS}	15.38	-3.95**	-1.76 ^{NS}
Gathuan dhan	11.08	-1.04**	-1.30 ^{NS}	22.63	-2.04**	-1.76 ^{NS}
Raja bagla	8.68	-1.15**	-1.30 ^{NS}	21.96	8.41*	-1.75 ^{NS}
Brown Rice-1	11.74	0.75*	-1.30 ^{NS}	21.89	-1.07**	-1.76 ^{NS}
Munibhog	10.65	-2.55**	-1.30 ^{NS}	25.58	7.64*	-1.76 ^{NS}
Danighoda	12.11	4.11**	-1.30 ^{NS}	23.00	2.14*	-1.76 ^{NS}
Saraitolia	10.14	2.84**	-1.30 ^{NS}	22.51	0.66*	-1.76 ^{NS}
Pangudi Goindi	9.80	3.24**	-1.30 ^{NS}	22.63	1.33*	-1.76 ^{NS}
Safri-17	10.75	3.47**	-1.30 ^{NS}	19.78	7.64*	-1.76 ^{NS}
Dubraj	10.61	2.49*	-1.30 ^{NS}	21.35	4.79*	-1.76 ^{NS}
Byalo	11.61	-2.84**	-1.30 ^{NS}	25.63	6.22*	-1.76 ^{NS}
Badshahbhog	15.99	-2.60**	-1.30 ^{NS}	23.98	-2.85**	-1.76 ^{NS}
Jonyaphool	10.43	-0.93**	-1.30 ^{NS}	23.71	9.22*	-1.75 ^{NS}
Mohlyanbanko	11.94	2.26*	-1.30 ^{NS}	19.50	-4.40**	-1.76 ^{NS}
Kanakbhog	9.08	-1.48**	-1.30 ^{NS}	17.63	-3.53**	-1.76 ^{NS}
Shwet Ganga	10.60	-1.05**	-1.30 ^{NS}	21.84	3.33*	-1.76 ^{NS}
Matko Dhan	11.84	-3.30**	-1.30 ^{NS}	21.20	10.91*	-1.75 ^{NS}
Suapankhi	12.89	16.72**	-1.29 ^{NS}	20.89	2.40*	-1.76 ^{NS}
Jhimiprass	11.26	1.10 ^{NS}	-1.30 ^{NS}	25.56	2.70*	-1.76 ^{NS}
Jauphool	11.25	-0.23**	-1.30 ^{NS}	19.00	7.34*	-1.76 ^{NS}
Kalajeera	11.71	-0.96**	-1.30 ^{NS}	23.31	-1.60**	-1.76 ^{NS}
Chhindmauri	16.36	7.12**	-1.30 ^{NS}	24.85	1.02 ^{NS}	-1.76 ^{NS}
Motipeera	10.25	-0.69**	-1.30 ^{NS}	22.47	-1.45**	-1.76 ^{NS}

Pedgadi	11.36	-1.65**	-1.30 NS	23.58	2.36*	-1.76 NS
Sonagathi	10.33	2.43*	-1.30 NS	18.56	3.01*	-1.76 NS
Jana Dhan	10.93	-3.47**	-1.30 NS	19.15	8.46*	-1.75 NS
Rela Dhan	9.96	-1.11**	-1.30 NS	21.27	1.18 NS	-1.76 NS
Ram Shree	10.98	4.04**	-1.30 NS	16.87	2.21*	-1.76 NS
Kari Alcha	9.57	1.41*	-1.30 NS	22.59	-3.71**	-1.76 NS
Jhilli Safri	11.56	0.06*	-1.30 NS	21.61	4.13*	-1.76 NS
Siyar	13.86	5.03**	-1.30 NS	21.73	2.24*	-1.76 NS
Karhani	11.41	-6.99**	-1.30 NS	22.31	-0.80**	-1.76 NS
Nandel	11.43	3.82**	-1.30 NS	21.89	-0.56**	-1.76 NS
Safri	10.73	-2.07**	-1.30 NS	22.16	1.78*	-1.76 NS
Dhaura Mundariya	9.45	-0.90**	-1.30 NS	15.78	-3.16*	-1.76 NS
Karhani-2	10.06	-0.91**	-1.30 NS	21.08	-1.59**	-1.76 NS
Sanchuriya	9.88	4.03**	-1.30 NS	18.03	-0.10**	-1.76 NS
Alsakar	11.68	4.36**	-1.30 NS	21.33	-2.53**	-1.76 NS
Luchai	11.18	-0.56**	-1.30 NS	20.99	-0.67**	-1.76 NS
Mai Dubraj	10.75	5.90**	-1.30 NS	21.91	-1.07**	-1.76 NS
TCDM-1	16.24	15.0**	-1.30 NS	24.04	-5.04 NS	-1.21 NS
Vikram-TCR	11.40	-0.46**	-1.30 NS	18.34	0.05**	-1.76 NS
CG Jawaphool Trombay	10.32	-2.96**	-1.30 NS	19.70	3.06*	-1.76 NS
Alsakar Mutant-3	11.62	2.57*	-1.30 NS	16.80	1.03 NS	-1.76 NS
Sanchuriya Mutant-6	12.42	5.12**	-1.30 NS	17.36	1.66*	-1.76 NS
Safri-17-5 Mutant	10.80	-1.85**	-1.30 NS	18.41	-2.22***	-1.76 NS
Safri-17-2-48 Mutant	21.51	3.15*	-1.30 NS	18.53	-2.93**	-1.76 NS
Luchai Mutant-1	12.71	-5.36**	-1.30 NS	21.83	-0.07**	-1.76 NS
Dubraj Sel-1	10.58	-0.38**	-1.30 NS	22.13	-4.56**	-1.76 NS
CG Zinc Rice-1	9.01	-3.22**	-1.30 NS	22.09	1.02 NS	-1.76 NS
CG Zinc Rice-2	12.67	1.16 NS	-1.26 NS	22.83	-2.06 NS	-1.60 NS
Zinco Rice MS	11.00	-4.95**	-1.30 NS	19.36	2.29*	-1.76 NS
Rajeshwari	13.76	-10.50**	-1.30 NS	21.51	2.82*	-1.76 NS
Protazin	12.73	0.36**	-1.30 NS	19.21	-1.63**	-1.76 NS
Grand mean	11.63			21.17		

in the current study. However, the seasonal variations for these elements were observed as reported earlier (Dixit *et al.,* 2019; Suman *et al.,* 2021). The variation in values may arise due to properties of grain such us relative position on the panicle (Su *et al*., 2014), moisture content (Rao *et al*., 2014), number of aleurone layer (Sellappan *et al*., 2009), etc.; soil properties such as pH, organic carbon content and inherent availability of iron and zinc in the soil (Chandel *et al*., 2010). Furthermore, high significant interaction effect of genotype x environment on the content of these micronutrients has been reported by Chandel *et al.* (2010); and Suwarto (2011). In the current study, iron and zinc contents were found high in landraces and similar results were observed and reported earlier (Anandan *et al.,* 2011; Nachimuthu *et al.,* 2014; Parikh *et al*., 2019). Sanjeeva *et al.,* (2020) experimented on various sets of germplasm and reported that 99 genotypes from germplasm and 344 lines from mapping populations exhibited ≥ 28 mg/kg zinc content in polished rice, meeting the target zinc content set by HarvestPlus.

In this study, Safri mutant 17-2-48 has the highest iron content, a newly developed mutant from Safri 17 local variety. This mutant can be directly released as a new variety with high iron content. Munibhog and Loktimachi were identified for the highest zinc content. Thus, these genotypes open the possibilities for the exploitation as a donor in biofortification breeding programs and the identification of genomic positions associated with iron and zinc contents in grains. Though the variation in micronutrient content depends on several factors, the germplasm stock in rice has sufficient variation to exploit for developing stable lines. As micronutrient malnutrition poses a significant global challenge, the development of

micronutrient enriched genotypes serves as the need of the hour. The top-performing genotypes identified in this research will be useful for selecting and breeding lines with enriched micronutrient status.

A stable variety for grain nutrition quality is an important consideration for biofortification. The genotype, environment, linear genotype X environment and non-linear genotype X environment interaction when tested against pooled deviation, showed significance for both iron and zinc content in the brown rice. Similar significance for zinc and iron content have earlier been reported by Oikeh *et al*., (2004), Prasanna *et al*., (2011), Suwarto (2011), Velu *et al*., (2012) and Ajmera *et al*., (2017). For iron content the genotypes Jhimiprass, Loktimachi and CG Zinc Rice 2 did not deviate from unity of regression, showing their stable and predictable performance. Therefore, they can be recommended for growing in all environments ranging from optimum to marginal environments. Similarly, for Zinc content the genotypes Rela Dhan, Chhindmauri and CG Zinc Rice 1 did not deviate significantly from the unity of regression. Therefore, these genotypes can be grown in any environment to get more or less stable performance in terms of the Zinc content in brown rice. Genotypes 32 and 27 recorded significant deviation from unity and less than unity ($b < 1$) for Iron and Zinc content, respectively, showing their below-average performance. These genotypes cannot take full advantage of the favorable conditions when grown in the optimum environment. Therefore, they are suitable for cultivation in marginal or suboptimal environments such as *Rabi* season. However, 23 and 25 genotypes for iron and zinc content have recorded significant deviation from unity and more than unity ($b > 1$). When grown in the optimum environment with the most suitable condition, such genotypes will result in above-average performance in terms of the micronutrient content in brown rice. Therefore, to take the best advantage, these varieties can be grown in *Kharif* season at regions having good soil fertility.

Conclusion

Existing genetic variation in rice germplasm offers scope for developing nutrient-rich varieties. Notably, there was an ample difference in Fe and Zn content suggesting the existence of genetic potential to increase the concentration of these micronutrients in rice grain. Micronutrient-rich rice genotype *viz*., Safri-17-2-48 Mutant, Loktimachi, Chhindmauri, Badsahbhog, Rudra, Danighoda, Suapankhi, TCDM-1, and Luchai Mutant-1 identified through this study may be further utilized in the breeding program as a donor for development of nutrient-rich or biofortified rice varieties. Additionally, genotypes Jhimiprass, Loktimachi, and CG zinc Rice 2 for iron content and Rela Dhan, Chhindmauri, and CG zinc rice 1 for zinc content exhibited an average response to the environment, making them suitable for all seasons.

Acknowledgment

We sincerely thank IRRI-SA Hub, ICRISAT campus, Hyderabad, India for providing the facility for iron and zinc estimation.

References

Aggett PJ (2020) Iron. In: Present Knowledge in Nutrition (Eleventh Edition), BP Marriott, DF Birt, VA Stallings and AA Yates (Ed.), Academic Press, USA, p 375–392.

Ajmera S, SS Kumar and V Ravindrababu (2017) Genotype × Environment interactions and stability analysis for grain iron and zinc concentrations in Rice (*Oryza Sativa* L.)Genotypes. *Int. J. Curr. Microbiol. App. Sci*. **6(7):** 1902-1913.

Anandan A, G Rajiv, R Eswaran, and M Prakash (2011) Genotypic variation and relationships between quality traits and trace elements in traditional and improved rice (*Oryza sativa* L.) genotypes. *J. Food Sci*. **76(4):** H122–H30.

Arora S, S Jood, and N Khetarpaul (2010) Effect of germination and probiotic fermentation on nutrient composition of barley-based food mixtures. *Food Chem*. **119(2):** 779-784.

Chandel G, S Banerjee, S See, R Meena, DJ Sharma and SB Verulkar (2010) Effects of different nitrogen fertilizer levels and native soil properties on rice grain Fe, Zn and protein contents. *Rice Sci*. **17(3):** 213–227.

Dixit S, UM Singh, R Abbai, T Ram, VK Singh and A Paul (2019) Identification of genomic region(s) responsible for high iron and zinc content in rice. *Sci. Rep*. **9:** 8136. doi: 10.1038/s41598-019-43888-y

Eberhart SA and WA Russell (1966) Stability parameters for comparing varieties. *Crop Sci*. **6(1):** 36- 40.

Kobayashi T, AJ Nagano and NK Nishizawa (2021) Iron deficiency-inducible peptide-coding genes OsIMA1 and OsIMA2 positively regulate a major pathway of iron uptake and translocation in rice. *J. Exp. Bot*. **72:** 2196–2211.

Kong D, SA Khan, H Wu, Y Liu and Ling HQ (2022) Biofortification of iron and zinc in rice and wheat. *J Integr Plant Biol.* **64(6):** 1157-67.

Lu L, S Tian, H Liao, J Zhang and X Yang (2013) Analysis of Metal Element Distributions in Rice (*Oryza sativa* L.) Seeds and Relocation during Germination Based on X-Ray Fluorescence Imaging of Zn, Fe, K, Ca, and Mn. *PLoS One*. **8(2):** e57360.

Maret W and HH Sandstead (2006) Zinc requirements and the risks and benefits of zinc supplementation. *J. Trace Elem. Med. Biol.* **20(1):** 3-18.

Nachimuthu VV, S Robin, D Sudhakar, S Rajeswari, M Raveendran, K Subramanian, S Tannidi, BA Pandian (2014) Genotypic Variation for Micronutrient Content in Traditional and Improved Rice Lines and its Role in Biofortification Programme Indian*J. Sci. Tech*. **7(9):** 1414–1425.

Oikeh SO, A Menkir, BM Dixon, RM Welch, RP Glahn and G Gauch (2004) Environmental stability of iron and zinc concentrations in grain of elite early maturing tropical maize genotypes grown under field conditions. *J. Agric. Sci.* **142:** 543–51.

Olsen LI and MG Palmgren (2014) Many rivers to cross: the journey of zinc from soil to seed. *Front. Plant Sci*. **5:** 30.

Omary MB, C Fong, J Rothschild and P Finney (2012) Effects of germination on the nutritional profile of gluten-free cereals and pseudo cereals: a review. *Cereal Chem*. **89(1):** 1-14.

Parikh M, AK Sarawgi, D Rao and B Sharma (2019) Assessment of genotypic variability for grain zinc and iron content in traditional and improved rice genotypes using energy

dispersive X-ray fluorescence spectrophotometer (ED-XRF). *Int. J. Curr. Microbiol. App. Sci.* **7(1):** 1967-1974.

Prasanna BM, S Mazumdar, M Chakraborti, F Hossain, KM Manjaiah, PK Agrawal, SK Guleria and HS Gupta (2011) Genetic variability and genotype × Environment interactions for kernel Iron and Zinc concentrations in maize (*Zea mays*) genotypes. *Indian J. Agric. Sci.* **81(8):** 704–11.

Pyo E, BL Tsang and ME Parker (2022) Rice as a vehicle for micronutrient fortification: A systematic review of micronutrient retention, organoleptic properties, and consumer acceptability. *Nutr. Rev.* **80:** 1062–1085.

Rao DS, PM Babu, P Swarnalatha, S Kota, VP Bhadana, GS Varaprasad and VR Babu (2014) Assessment of grain zinc and iron variability in rice germplasm using Energy Dispersive X-ray Fluorescence Spectrophotometer. *J. Rice Res*. **7(1):** 45-52.

Roy SC and BD Sharma (2014) Assessment of genetic diversity in rice (*Oryza sativa* L.) germplasm based on agro-morphology traits and zinc-iron content for crop improvement. *Physiol. Mol. Biol. Plants,* **20(2):** 209–24.

Ryu MS and TB Aydemir (2020) Zinc. In: *Present Knowledge in Nutrition (Eleventh Edition)*. BP Marriott, DF Birt, VA Stallings, AA Yates (Ed.), Academic Press, USA, p 393–408.

Sanjeeva RD, CN Neeraja, BP Madhu, B Nirmala, K Suman, LV Rao, K Surekha, P Raghu,T Longvah, P Surendra and R Kumar (2020) Zinc biofortified rice varieties: challenges,possibilities, and progress in India. *Front. Nutr.* **7:** 26.

Sellappan K, K Datta, V Parkhi and SK Datta (2009) Rice caryopsis structure in relation to distribution of micronutrients (iron, zinc, b-carotene) of rice cultivars including transgenic indica rice. *Plant Sci.* **177:** 557–562.

Shahzad Z, H Rouached and A Rakha (2014) Combating mineral malnutrition through iron and zinc biofortification of cereals. *Compr. Rev. Food Sci. Food Saf.* **13(3):** 329-346.

Su D, F Sultan, NC Zhao, BT Lei, FB Wang, G Pan and FM Cheng (2014) Positional variation in grain mineral nutrients within a rice panicle and its relation to phytic acid concentration. *J. Zhejiang Univ. Sci. B.* **15(11):** 986-996.

Suman K, CN Neeraja, P Madhubabu, S Rathod, S Bej, KP Jadhav, JA Kumar, U Chaitanya, SC Pawar, Rani SH, LV Subbarao and SR Voleti (2021) Identification of promising RILs for high grain zinc through genotype x environment analysis and stable grain zinc QTL using SSRs and SNPs in rice (Oryza sativa L.). *Front. Plant Sci.* **12:** 587482.

Suwarto N (2011) Genotype×Environment Interaction for Iron Concentration of Rice in Central Java of Indonesia. *Rice Sci*. **18(1):** 75–78.

Velu G, RP Singh, J Huerta-Espino, RJ Pena, B Arun, MA Singh, YM Mujahide, VS Sohuf, GS Mavif, J Crossaa, G Alvarado, AK Joshi and WH Pfeiffer (2012) Performance of biofortified spring wheat genotypes in target environments for grain zinc and iron concentrations. *Field Crops Res.* **137:** 261–267.

Zhang GM, TQ Zheng, Z Chen, YL Wang, Y Wang, YM Shi, CC Wang, LY Zhang, JT Ma, LW Deng, W Li, TT Xu, CZ Liang, JL Xu and ZK Li (2018) Joint exploration of favorable haplotypes for mineral concentrations in milled grains of rice (*Oryza sativa* L.). *Front. Plant Sci*. **9:** 447.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2023.00036.2.11

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Morphological and Molecular Analyses of Grain Traits in Aromatic Rice Landrace Accessions from Indo-Gangetic Plain Region of India

Rakesh K. Bairwa[1,2], Mahesh C. Yadav[1*], Gopala K. Subbaiyan[3], Amar K. Kushwaha[3] and Monika A. Joshi[4]

Abstract

Information on genetic variation in rice grain's morphological traits is vital, affecting its yield and quality parameters. In present study, 92 accessions of aromatic rice landrace germplasm from the Indo-Gangetic plain region of India have assessed for grain morphometric traits as-well-as for genotypic mutation in *exon* 3 of grain size (*GS3*) gene. Significant variability was detected for the grain traits, namely rough rice length and breadth (RRL, RRB), rough rice length/breadth ratio (RRLB), 1000-grain weight (TGW), brown rice length and breadth (BRL, BRB), and brown rice length/breadth ratio (BRLB). The highest values of the phenotypic coefficient of variation and genotypic coefficient of variation were observed for TGW (40.56; 38.38%) and the lowest for BRB (14.96; 15.02%). For all the grain traits, high heritability (broad-sense) was observed, which ranged from 85.67 (BRLB) to 99.13% (BRB). Genetic advances ranged from 0.58 (BRB) to 9.13 (TGW). Principal component (PC) analysis revealed that first two PCs explained 86.04% of phenotypic variation. A positive association found between all the grain traits except grain breadth (RRB and BRB), which showed a negative correlation with the grain length-breadth ratio. The variation revealed for C/A single nucleotide polymorphism in *GS*3 gene. The superior accessions for grain traits, namely RRB (Jeera Sail/IC419047), TGW (Hansraj/IC0419006) and BRL (Kala Jira/IC0419052) identified. The genetically variable germplasm of aromatic rice with superior grain traits could be utilized for genetic enhancement of grain size in aromatic rices.

Keywords: Aromatic rice landraces, Characterization, Grain size, GS3 gene, Indo-Gangetic Plains.

[1]National Bureau of Plant Genetic Resources, ICAR- New Delhi, Delhi, India.

[2]Division of Genomic Resources, ICAR- New Delhi, Delhi, India.

[3]Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, Delhi, India.

[4]Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi, Delhi, India.

***Author for correspondence:**

mahesh.yadav1@icar.gov.in

Received: 01/12/2022 **Revised:** 15/12/2022

Accepted: 16/12/2022

How to cite this article: Bairwa, R.K., Yadav, M.C., Subbaiyan, G.K., Kushwaha, A.K., Joshi, M.A. (2023). Morphological and Molecular Analyses of Grain Traits in Aromatic Rice Landrace Accessions from Indo-Gangetic Plain Region of India. *Indian J. Plant Genetic Resources*. 36(2), 290-300.

DOI: 10.5958/0976-1926.2023.00036.2.11

Introduction

Rice is known as Global grain because it is the primary source of food for half of the global population (Brar and Khush, 2018). It fulfils nearly 19% of the global population's calorie requirements per capita and 13% of their protein needs. In terms of global rice production, India ranks second. Indian region represents the center of origin and diversity for aromatic rice. Aromatic rice possesses superior grain and cooking quality and has aroma mainly due to the presence of 2-acetyl pyrroline (Buttery *et al*., 1983; Verma and Srivastav, 2022). These rices have better nutritional qualities and hence fetch premium price than the non-fragrant types. The Indian subcontinent is gifted with an enormous diversity of aromatic rice landraces; presently, there are hundreds of locally adapted genotypes of aromatic rices (Khush, 2000). Indian aromatic rices exhibit variation for grain length from small, medium to long grain types having mild to strong aroma (Singh *et al*., 2000). In rice, crop yield is primarily determined by three characters: number of panicles per plant, the number of grains per panicle and 1000 grain weight (TGW). TGW is positively associated with grain size. Grain size is positively correlated with grain length, width, and thickness (Xu *et al*., 2002; Zeng *et al*., 2017). Large seeds are favored

during domestication due to ease of harvesting and better seedling vigour (Harlan *et al*., 1973). Rice exhibits substantial phenotypic diversity for seed size and shape unlike other cereal crops. In rice, cultivated varieties showed greater seed length variation than wild rice, probably due to human selection (Takano-Kai *et al*., 2009). In rice breeding, grain size is an important agronomic trait. In rice, breeders tend to select large-seeded plants for better yield and appropriate grain-sized plants for milling and market preferences (Wang *et al*., 2011). Market value and consumer preference in rice are mainly driven by rice seed shape and appearance. Grains with long and slender shape are preferred by peoples of India, Pakistan, Thailand, China and the United States, while short and bold-grained rice varieties are consumed by peoples of Japan, South Korea and Sri Lanka (Juliano and Villareal, 1993; Unnevehr *et al*., 1992). Grain size also plays a pivotal role in the adoption of new varieties (Champagne *et al*., 1999). However, breeding for enhanced grain size is a tedious task, because of its quantitative inheritance (McKenzie and Rutger, 1983). In rice, several QTLs governing seed size have been identified. Few of them have been successfully cloned and functionally validated *viz*., *GS3* (Fan *et al*., 2006), *GW2* (Song *et al*., 2007), *GS5* (Li *et al*., 2011), *GS7* (Shao *et al*., 2012), *qGS*10 (Zhu *et al*., 2019) and *qTGW*2 (Ruan *et al*., 2020). Among these genes, *GS*3 is the major gene governing the grain size in rice. One common non-sense mutation (C/A SNP) detected at the second exon of the *GS3* gene results in change of cysteine codon (TGC) to termination codon (TGA). This premature termination resulted in a 178-aa truncation in the C-terminus of the predicted protein in the large-grain size group of rice varieties. *GS3* act as a negative regulator of grain size. The wild allele is present in small to medium grain rice genotypes and the mutant allele in large grain size genotypes (Fan *et al*., 2006). Till date most of the breeding efforts were focused on Basmati-type aromatic rice. But Indian region is blessed with hundreds of short to medium grained indigenous aromatic landraces, adapted to different regions' local climatic conditions. Basmati cultivation is confined to northern states of India, while these aromatic landraces are under cultivated throughout the country. Although aromatic rice landraces possess rich diversity with respect to grain dimension traits, there have been limited efforts to characterize them for grain traits. In context of this, the current study was designed to characterize 92 accessions of aromatic rice landraces for grain features. This study revealed the high extent of variability for grain traits and allelic variation in *GS*3 gene, which could be useful for mining novel alleles for grain quality traits.

Materials and Methods

Germplasm Materials and Field Experimentation

A panel of 92 aromatic rice landrace accessions (Table 1), representing 37 districts and 10 states of Indian Union were selected for the study (Table 2). Among these, 86 accessions were from the Indo-Gangetic plains of India, representing centre of diversity for aromatic rices. The seeds of aromatic rice landraces were obtained from Indian National Genebank housed at ICAR-NBPGR, New Delhi. Geo-referencing of the aromatic rice landrace accessions was performed using passport data information using 'DIVA-GIS' software version 7.5 (Hijmans *et al*., 2001; Figure 1). The rice accessions were grown under normal sown irrigated conditions at Experimental Farm of Division of Genetics, ICAR-IARI, New Delhi during *Kharif* 2017. The seeds were sown in a flat-bed nursery. After 25 days, the seedlings were transplanted under Augmented Block Design with four standard checks: Badshahbhog, Kalanamak, P1176, and Taraori Basmati using six replications. The plant-to-plant and row to row distances were 15 and 20 cm, respectively. Standard package of practices were used to raise a healthy crop of rice landraces.

Morphological Characterization

After harvesting individual aromatic rice accessions, grains were shade dried to reduce the moisture content to 12% level and kept in zip-lock bags at room temperature. Grain's phenotypic observations were recorded for seven quantitative and two qualitative traits. Ten grains from each accession were measured for data recording of seven quantitative traits namely rough rice length (RRL), rough rice breadth (RRB), rough rice length to breadth ratio (RRLB), thousand grain weight (TGW), brown rice length (BRL), brown rice breadth (BRB) and brown rice length to breadth ratio (BRLB). The lemma and palea (husk) color and caryopsis pericarp color were recorded for all the landrace materials.

Molecular Analysis

Genomic DNA extracted from 30 random grains of each accession to access the allelic polymorphism in aromatic rice landrace accessions. The grains were germinated in folds of germination paper and DNA was extracted from two weeks old seedlings using cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). The quality and quantity of extracted DNA was checked in 0.8% agarose gel using standard uncut λ phage DNA as a weight marker. The DNA samples of 96 accessions of aromatic rices were diluted in Tris-EDTA (ethylene diamine tetra acetic acid) buffer to obtain a working concentration of 25 ng/µL. The rice landrace accessions were genotyped for grain length gene '*GS3*' located on chromosome 3 of the rice genome, using SF28 primer-pair (Fan *et al*., 2008; Figure 2). PCR amplification was carried out in 10 µL reaction mixture containing DNA template 25 ng, GoTaq Green (1X; Promega) containing optimized concentration of PCR buffer, dNTPs and enzyme Taq DNA polymerase, primers (20 pmol) and DNase free H_2O (Promega). The sequences of SF28 forward and reverse primers are 5'-TGCCCATCTCCCTCGTTTAC-3' and 5'-GAAACAGC AGGCTGGCTTAC-3'. The PCR was performed

Table 1: Details of aromatic rice landraces from Indo-Gangetic plains of India used in the assessment of genetic variation and allele mining of quality traits

S. No.	*Acc. Code*	*IC Number*	*Name of Landrace*	*Geographical Coordinates*	*District*	*State*
1.	ARG01	IC0086170	Adamchini	25° 1′ N 82° 33′ E	Mirzapur	Uttar Pradesh
2.	ARG02	IC0553809	Adamchini	25° 1′ N 82° 33′ E	Mirzapur	Uttar Pradesh
3.	ARG03	IC0553863	Adamchini	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
4.	ARG04	IC0553848	Adamchini	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
5.	ARG05	IC0553871	Adamchini	35° 0′ N 80° 0′ E	Chandauli	Uttar Pradesh
6.	ARG06	IC0553887	Adamchini	35° 0′ N 80° 0′ E	Chandauli	Uttar Pradesh
7.	ARG07	IC0554649	Kalanamak	26° 53′ N 83° 58′ E	Kushinagar	Uttar Pradesh
8.	ARG08	IC0574862	Kalanamak-1	25° 8′ N 83° 15′ E	Chandauli	Uttar Pradesh
9.	ARG09	IC0553882	Badshahbhog	35° 0′ N 80° 0′ E	Chandauli	Uttar Pradesh
10.	ARG10	IC0574865	Kalanamak-3	26° 39′ N 82° 0′ E	Ayodhya	Uttar Pradesh
11.	ARG11	IC0554631	Kalanamak	27° 18′ N 83° 43′ E	Maharajganj	Uttar Pradesh
12.	ARG12	IC0554622	Kalanamak	27° 16′ N 82° 49′ E	Siddharthnagar	Uttar Pradesh
13.	ARG13	IC0554644	Kalanamak	27° 8′ N 83° 33′ E	Maharajganj	Uttar Pradesh
14.	ARG14	IC0553819	Kalanamak	25° 1′ N 82° 33′ E	Mirzapur	Uttar Pradesh
15.	ARG15	IC0554657	Sakkarchini	27° 8′ N 81° 57′ E	Gonda	Uttar Pradesh
16.	ARG16	IC0554618	Kalanamak	28° 39′ N 79° 22′ E	Bareilly	Uttar Pradesh
17.	ARG17	IC0554624	Kalanamak	26° 48′ N 82° 45′ E	Basti	Uttar Pradesh
18.	ARG18	IC0553830	Badshahbhog	25° 8′ N 82° 33′ E	Mirzapur	Uttar Pradesh
19.	ARG19	IC0553833	Badshahbhog	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
20.	ARG20	IC0554639	Sakkarchini	27° 25′ N 82° 10′ E	Balrampur	Uttar Pradesh
21.	ARG21	IC0554641	Sakkarchini	27° 34′ N 81° 35′ E	Bahraich	Uttar Pradesh
22.	ARG22	IC0553836	Sonachur	25° 8′ N 82° 33′ E	Mirzapur	Uttar Pradesh
23.	ARG23	IC0370895	Sonachur	25° 18′ N 81° 57′ E	Prayagraj	Uttar Pradesh
24.	ARG24	IC0553875	Sonachur	35° 0′ N 80° 0′ E	Chandauli	Uttar Pradesh
25.	ARG25	IC0370882	Thakurbhog	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
26.	ARG26	IC0554652	Badshah Pasand	27° 34′ N 81° 35′ E	Bahraich	Uttar Pradesh
27.	ARG27	IC0554638	Badshah Pasand	26° 28′ N 81° 35′ E	Ambedkar Nagar	Uttar Pradesh
28.	ARG28	IC0554627	Lal Mati	26° 46′ N 81° 8′ E	Ayodhya	Uttar Pradesh
29.	ARG29	IC0554633	Lal Mati	26° 55′ N 81° 11′ E	Barabanki	Uttar Pradesh
30.	ARG30	IC0553883	Tulsi Manjari	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
31.	ARG31	IC0553844	Tulsi Manjari	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
32.	ARG32	IC0554629	Dubraj	26° 55′ N 81° 11′ E	Raibarelly	Uttar Pradesh
33.	ARG33	IC0553879	Shyam Jeera	24° 27′ N 82° 59′ E	Sonbhadra	Uttar Pradesh
34.	ARG34	IC0554647	Kanak Jeera	27° 16′ N 82° 49′ E	Siddharthnagar	Uttar Pradesh
35.	ARG35	IC0554654	Gopalbhog	26° 46′ N 83° 2′ E	Sant Kabir Nagar	Uttar Pradesh
36.	ARG36	IC0553839	Govindbhog	25° 8′ N 82° 33′ E	Mirzapur	Uttar Pradesh
37.	ARG37	IC0554646	Juhi Bengal	27° 17′ N 83° 5′ E	Siddharthnagar	Uttar Pradesh
38.	ARG38	IC0574876	Dhania 32	26° 39′ N 82° 0′ E	Ayodhya	Uttar Pradesh
39.	ARG39	IC0574922	Tulsi Joha	27° 12′ N 94° 9′ E	Lakhimpur	Assam
40.	ARG40	IC0574875	Juhi Bengal	26° 39′ N 82° 0′ E	Ayodhya	Uttar Pradesh
41.	ARG41	IC0419241	Tulsi Prasad	30° 54′ N 75° 48′ E	PAU, Ludhiana*	Punjab
42.	ARG42	IC0419239	Tulsi Joha-b	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
43.	ARG43	IC0419238	Tulsi Joha-a	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab

44.	ARG44	IC0418945	Chinnisakkar	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
45.	ARG45	IC0419046	Jeera Sail-a	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
46.	ARG46	IC0419047	Jeera Sail-b	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
47.	ARG47	IC0419219	Soribhog	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
48.	ARG48	IC0418906	Begami 1-T-1-A	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
49.	ARG49	IC0419006	Hansraj-a	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
50.	ARG50	IC0418918	Bindli sd mutant-a	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
51.	ARG51	IC0418919	Bindli sd mutant-b	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
52.	ARG52	IC0418920	Bindli sd mutant-c	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
53.	ARG53	IC0419052	Kala Jira-245	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
54.	ARG54	IC0419055	Kala Jira 449-b	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
55.	ARG55	IC0419050	Kala Jira-8-1-a	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
56.	ARG56	IC0419053	Kala Jira-286	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
57.	ARG57	IC0419051	Kala Jira-8-1-b	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
58.	ARG58	IC0419054	Kala Jira 449-a	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
59.	ARG59	IC0418979	Dulhabhog	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
60.	ARG60	IC0574897	Malbhog Sataria	25° 46′ N 85° 52′ E	Samastipur	Bihar
61.	ARG61	IC0569188	Champaran Basmati	27° 9′ N 84° 21′ E	West Champaran	Bihar
62.	ARG62	IC0574775	Tulsimanjari I	25° 20′ N 86° 58′ E	Bhagalpur	Bihar
63.	ARG63	IC0574776	Tulsimanjari II	25° 20′ N 86° 58′ E	Bhagalpur	Bihar
64.	ARG64	IC0574843	Kalanamak	25° 46′ N 85° 52′ E	Samastipur	Bihar
65.	ARG65	IC0574883	Malbhog Sataria-B	25° 46′ N 85° 52′ E	Samastipur	Bihar
66.	ARG66	IC0574927	Sonachur	25° 46′ N 85° 52′ E	Samastipur	Bihar
67.	ARG67	IC0574793	Badshahbhog	25° 20′ N 86° 58′ E	Bhagalpur	Bihar
68.	ARG68	IC0574830	Badshahbhog	25° 46′ N 85° 52′ E	Samastipur	Bihar
69.	ARG69	IC0569410	Kalonuniya	26° 32′ N 88° 43′ E	Jalpaiguri	West Bengal
70.	ARG70	IC0470970	Badshahbhog	22° 8′ N 88° 24′ E	South 24 Parganas	West Bengal
71.	ARG71	IC0569444	Tulsibhog	25° 58′ N 88° 3′ E	North Dinajpur	West Bengal
72.	ARG72	IC0569416	Lal Badshahbhog	23° 16′ N 86° 25′ E	Puruliya	West Bengal
73.	ARG73	IC0584173	Hari Bhog	23° 28′ N 88° 31′ E	Nadia	West Bengal
74.	ARG74	IC0594002	Kanakchur	22° 11′ N 88° 41′ E	South 24 Parganas	West Bengal
75.	ARG75	IC0594012	Sitabhog	22° 8′ N 88° 24′ E	South 24 Parganas	West Bengal
76.	ARG76	IC0594017	Tulaipanji	22° 11′ N 88° 48′ E	South 24 Parganas	West Bengal
77.	ARG77	IC0569443	Tulaipanji	26° 20′ N 89° 26′ E	Cooch Behar	West Bengal
78.	ARG78	IC0596840	Kalonunia	22° 13′ N 88° 46′ E	South 24 Parganas	West Bengal
79.	ARG79	IC0596834	Gobindabhog	22° 9′ N 88° 49′ E	South 24 Parganas	West Bengal
80.	ARG80	IC0596837	Gobindabhog	22° 20′ N 88° 41′ E	South 24 Parganas	West Bengal
81.	ARG81	IC0470934	Govindbhog	22° 8′ N 88° 24′ E	South 24 Parganas	West Bengal
82.	ARG82	IC0611186	Lal Basmati Local	31° 35′ N 76° 55′ E	Mandi	H.P.
83.	ARG83	IC0611190	Begami	32° 33′ N 76° 7′ E	Chamba	H.P.
84.	ARG84	IC0085729	Gandhasalai	12° 46′ N 75° 12′ E	Dakshina Kannada	Karnataka
85.	ARG85	IC0280808	Jeerakachampav	9° 51′ N 76° 57′ E	Idukki	Kerala
86.	ARG86	IC0591553	Wayanad Jeerakasala	11° 22′ N 76° 7′ E	Wayanad	Kerala
87.	ARG87	IC0520332	Jeerakasala	11° 51′ N 75° 21′ E	Kannur	Kerala
88.	ARG88	IC0121907	Ambemohar	21° 8′ N 79° 5′ E	Nagpur	Maharashtra
89.	ARG89	IC0574923	Tulsiamrit	17° 40′ N 74° 10′ E	Satara	Maharashtra

90.	ARG90	IC0618897	Jeeragasamba	9° 13′ N 78° 25′ E	Ramanathapuram	Tamil Nadu
91.	ARG91	IC0454228	Kamod	30° 54′ N 75° 48′ E	PAU, Ludhiana	Punjab
92.	ARG92	IC0553842	Tinsukhiya	25° 8′ N 82° 33′ E	Mirzapur	Uttar Pradesh
93.	ARG93	Check01	Badshahbhog	28° 38′ N 77° 10′ E	Genetics, IARI*	Delhi
94.	ARG94	Check02	Kalanamak	28° 38′ N 77° 10′ E	Genetics, IARI	Delhi
95.	ARG95	Check03	P1176	28° 38′ N 77° 10′ E	Genetics, IARI	Delhi
96.	ARG96	Check04	Taraori Basmati	28° 38′ N 77° 10′ E	Genetics, IARI	Delhi

*Abbreviations: PAU = Punjab agricultural University, Ludhiana; H.P. = Himachal Pradesh; Genetics IARI = Genetics Division of ICAR - Indian Agricultural Research Institute, New Delhi.

Table 2: Indian states, districts and number of aromatic rice landrace accessions characterized

S. No.	*Name of the state*	*Name of district and number of accessions*	*Number of collections*
(a).	*Indo-Gangetic Plain region (27 Districts)*		
1.	Uttar Pradesh	Mirzapur (7), Sonbhadra (7), Chandauli (5), Kushinagar (1), Ayodhya (4), Maharajganj (2), Siddharthnagar (3), Gonda (1), Bareilly (1), Basti (1), Balrampur (1), Bahraich (2), Ambedkar Nagar (1), Barabanki (1), Raibarelly (1), Sant Kabir Nagar (1) and Prayagraj (1)	40
2.	Punjab	Ludhiana (20)	20
3.	Bihar	Samastipur (5), Bhagalpur (3) and West Champaran (1)	9
4.	West Bengal	South 24 Parganas (8), Jalpaiguri (1), Uttar Dinajpur (1), Puruliya (1), Nadia (1) and Cooch Behar (1)	13
(b).	*Other States of India (10 Districts)*		
5.	Assam	Lakhimpur (1)	1
6.	Himachal Pradesh	Mandi (1) and Chamba (1)	2
7.	Karnataka	Dakshina Kannada (1)	1
8.	Kerala	Idukki (1), Wayanad (1) and Kannur (1)	3
9.	Maharashtra	Nagpur (1), Satara (1)	2
10.	Tamil Nadu	Ramanathapuram (1)	1

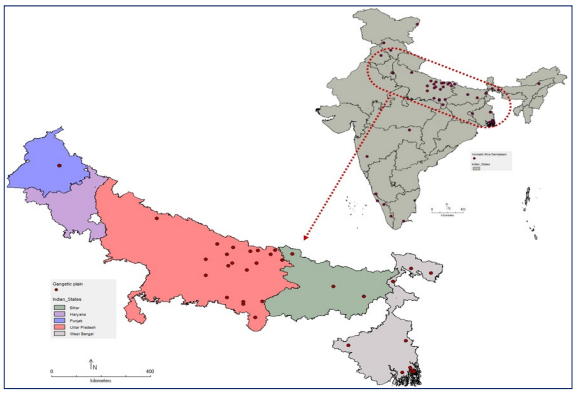

Figure 1: Geo-referencing of 92 accessions of aromatic rice landrace collection sites located in 37 districts of 10 states of India.

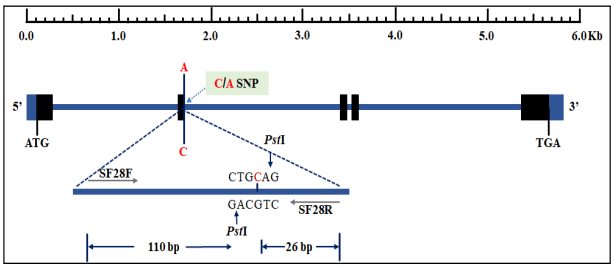

Figure 2: Structure of GS3 gene. Black boxes indicate exons, while Blue boxes indicate 5′ and 3′ Untranslated Regions. Functional mutation responsible for grain length is C/A SNP in the second exon (modified from Fan *et al.*, 2008).

in Eppendorf's thermocycler using the following thermal cycling parameters: initial DNA denaturation at 95°C for 4 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds and finally a primer extension cycle of 5 minutes at 72°C. Restriction digestion was performed in 15 µL reaction volume containing buffer (O), *Pst*I enzyme, and H_2O (Promega) to digest *GS3* gene PCR amplicons. The reaction mixture was incubated for 60 minutes at 37°C for complete digestion. The enzyme-cleaved products were separated in 3.5% agarose gel pre-stained with ethidium bromide. The resolved products were visualized under UV light and documented using the syngene gel documentation system. The gel profiles were scored for the presence of domesticated and mutated alleles of *GS3* gene.

Statistical Analysis

The data of seven quantitative grain traits were subjected to analysis of variance (ANOVA). Descriptive statistics and genetic parameters were calculated. The adjusted mean

values obtained from ANOVA were used for principal component analysis (PCA) and for correlation studies. Pearson correlation coefficient values were used to determine the relationship between different traits. The R statistical environment (base package) has been used for the analyses. For two qualitative qualities, frequency distribution bar diagrams were generated. Sivasubramanian and Madhavamenon (1973) categorization was used to classify the PCV and GCV values, whereas Robinson (1966) categorization of heritability values was followed. Genetic advance was categorized in accordance with Johnson *et al*. (1955).

Results

Phenotypic Diversity for Quantitative Grain Traits

ANOVA showed significant differences for all the grain traits and revealed the presence of high extent of morphological variability in the aromatic rice landraces (Supplementary Table 1). The descriptive statistics for seven grain morphometric characters revealed that ample variability exists in aromatic rice landrace germplasm (Table 3 Figure 3). The significant variations were observed for RRL, which ranged from 5.04 (IC0553830/ Badshahbhog) to 10.77 mm (Taraori Basmati) with mean value of 6.91 mm, and RRB, which varied from 1.61 (IC0554622/ Kalanamak) to 3.45 mm (IC419047/Jeera Sail-b) with mean value of 2.18 mm. RRLB ranged from 2.07 (IC0419219/ Soribhog) to 5.25 mm (Taraori Basmati) with average of 3.12 mm. TGW ranged from 4.98 (IC0569444/Tulsibhog) to 27.68 g (IC0419006/ Hansraj-a) with an average of 12.18 g. BRL varied from 3.5 (IC0553809/ Adamchini) to 7.69 mm (IC0419052/ Kala Jira-245) with mean value of 4.92 mm, whereas BRB ranged from 1.49 (IC0553819/ Kalanamak) to 3.13 mm (IC0419047/Jeera Sail) with mean value of 1.88 mm. For BRLB, the mean value was 2.63 on a scale of 1.72 (IC0419047/Jeera sail) to 4.52 (Taraori Basmati). Medium values of GCV and PCV were observed for five traits *viz*., RRL, RRB, BRL, BRB and BRLB, while high GCV and PCV values were recorded for TGW and RRLB. All the traits under study had comparable PCV and GCV values, indicating lesser influence of the environment on the expression of these traits. The broad-sense heritability estimates ranged from 85.7 (BRLB) to 99.1% (BRB) with all traits showing high heritability. The genetic advance ranged from 30.72 (BRB) to 74.92% (TGW) revealing high GA for all the traits.

Ward's method of agglomerative cluster analysis grouped 92 aromatic rice landrace accessions into three clusters based on Euclidean distances (Figure 4). The clusters I, II and III comprised 26, 37 and 33 aromatic rice landrace accessions. The cophenetic value of 0.497 was obtained for the dendrogram.

Principal Component Analysis

Principal component analysis (PCA) revealed that first two PCs accounted for 86.04% variance. PCA bi-plot illustrated the distribution and diversity type for grain morphometric traits and the aromatic rice landrace accessions (Figure 5). The diagram depicts that all grain morphometric traits had substantial diversity. On the basis of PCA bi-plot aromatic rice landrace accessions could be selected for various grain traits like accession IC0419006 (Hansraj) for TGW and Taraori Basmati for BRL and BRLB ratio. PC1 contributed to 55.34% of the total variation with large loadings in BRL (0.4954), RRL (0.4916), TGW (0.4394) and BRLB (0.3753). The PC2 contributed 30.70% of the overall variation with large contributions from RRB (0.5505), BRB (0.5493), RRLB (0.4135) and BRLB (0.3962). The PC3, PC4 and PC5 contributed 8.06, 3.05 and 1.88% of the total variance, respectively (Supplementary Table 2). In the present study, total variance existing among aromatic rice landraces represented by seven PCs and the first three PCs contributed to 94.10% of the total variance, indicating a high correlation among the traits being investigated. Scree plot depicted the percentage proportion of variance associated with each PC (Figure 6). PC1 contributed to 55.34% variability of total data set. The variance is gradually declining up to PC3. After

Table 3: Descriptive statistics of seven quantitative grain traits analysed in 96 accessions of aromatic rices

Trait	Range		Mean ± SE	SD	CV	GCV	PCV	ECV	Hbs (%)	GA	GAM
	Min.	Max.									
RRL	5.04	10.77	6.91 ± 0.13	1.32	2.41	18.1	18.27	2.48	98.16	2.56	36.99
RRB	1.61	3.45	2.18 ± 0.04	0.36	3.50	16.41	16.77	3.46	95.75	0.72	33.13
RRLB	2.07	5.25	3.12 ± 0.08	0.77	5.11	23.02	23.63	5.34	94.89	1.45	46.26
TGW	4.98	27.68	12.18 ± 0.49	4.84	12.8	38.38	40.56	13.12	89.54	9.13	74.92
BRL	3.50	7.69	4.92 ± 0.10	1.02	5.69	19.63	20.47	5.80	91.97	1.91	38.85
BRB	1.49	3.13	1.88 ± 0.03	0.28	1.43	14.96	15.02	1.40	99.13	0.58	30.72
BRLB	1.72	4.52	2.63 ± 0.05	0.53	6.85	17.44	18.85	7.13	85.67	0.88	33.31

Abbreviations: RRL: Rough rice length, RRB: Rough rice breadth, RRLB: Rough rice length to breadth ratio, TGW: Thousand grain weight, BRL: Brown rice length BRB: Brown rice Breadth; BRB: Brown rice length to breadth ratio PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, Hbs: Heritability (broad sense), GA: Genetic advance, GAM: Genetic advance as per cent of mean.

Figure 3: Variability of husk color in representative accessions of aromatic rice landraces a): Adamchini (ARG06), b): Badshahbhog (ARG18), c): Sakkarchini (ARG 21), d): Lal Mati (ARG29), e): Kanak Jeera (ARG34), f): Gopalbhog (ARG35), g): Kalanamak (ARG39), h): Tulsi Prasad (ARG41), i): Tulsi Joha (ARG42), j): Jeera Sail (ARG46), k): Soribhog (ARG47) and l): Bindli (ARG50). Below panel: variability for brown color of dehusked grains (caryopsis pericarp).

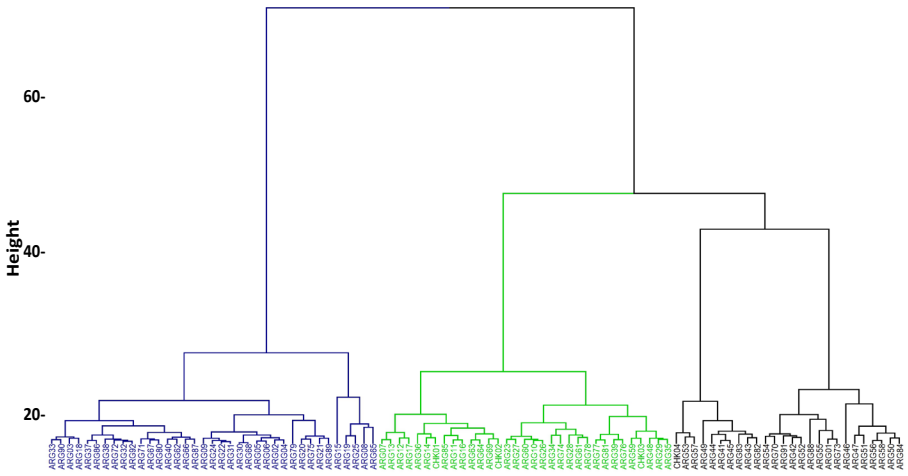

Figure 4: Cluster dendrogram constructed using Ward.D2 method of hierarchical clustering showing inter-relationships amongst 96 accessions of aromatic rice accessions. Three clusters formed.

that straight line with little variance was observed in each PC. From the graph, we could conclude that maximum variation was included in PC1 and PC2; hence, the genotypes could be selected from these PCs for further breeding programmes.

Correlation Studies

Understanding the relationship between grain traits is important for improving yield and quality of rice. To comprehend the type and extent of the association between the seven quantitative grain traits, Pearson's correlation coefficients were calculated (Supplementary Table 3; Figure 7). RRL had highly significant positive correlation with BRL (0.94), BRLB (0.76) and RRLB (0.61). RRB showed highly significant positive association with BRB (0.63). RRLB ratio

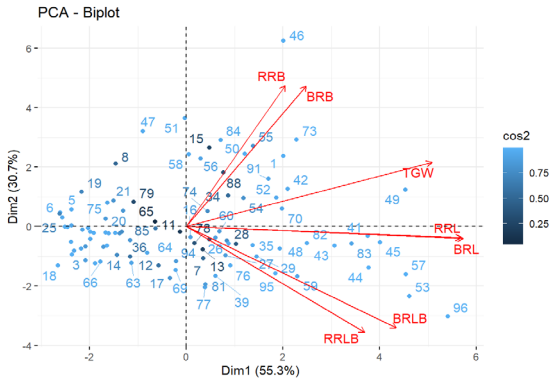

Figure 5: Principal Component-Biplot depicting two dimensional alignment of aromatic rice landrace accessions for seven quantitative grain traits for first two principal components (PC1 and PC1).

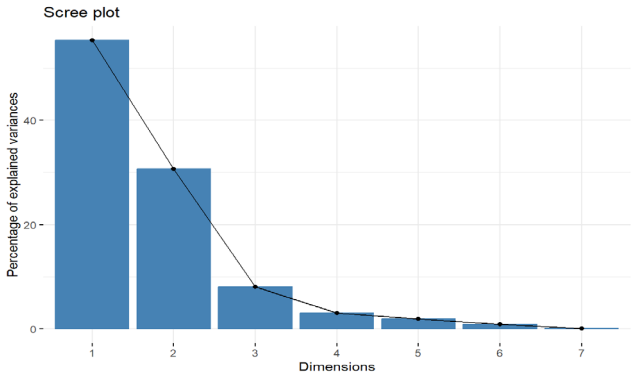

Figure 6: Scree plot for different PCs in aromatic rice germplasm accessions.

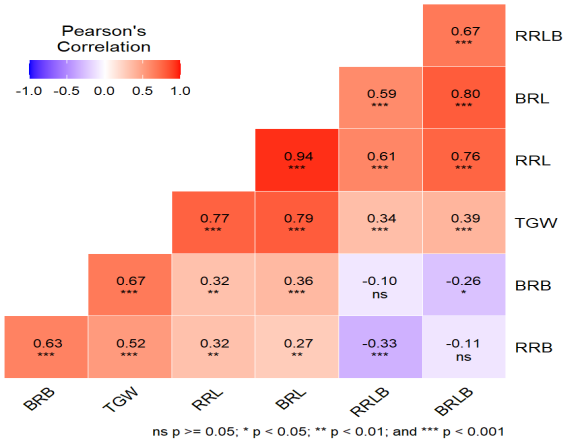

Figure 7: Correlation between rice grain dimension traits. Rough rice length (RRL), rough rice breadth (RRB), rough rice length to breadth ratio (RRLB), brown rice length (BRL), brown rice breadth (BRB), brown rice length to breadth ratio (BRLB) and thousand grain weight (TGW).

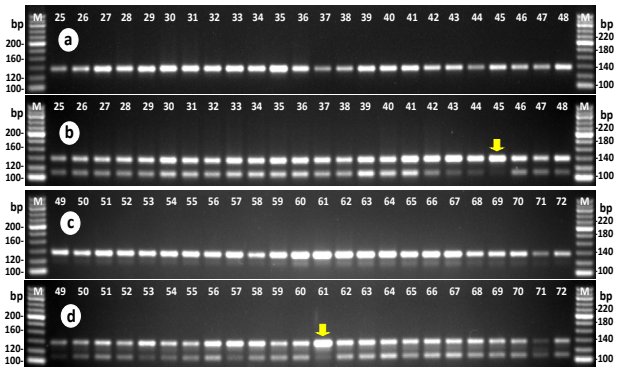

Figure 8: Gel profile of GS3 gene generated by SF28 primers in 96 aromatic rice accessions. (a) Undigested amplicons of 136 bp in accessions 25-48, (b) DNA fragments of 136 bp and 110 bp after digestion with PstI restriction enzyme in accessions 25-48, (c) Undigested amplicons of 136 bp in accessions 49-72, and (d) DNA fragments of 136 bp and 110 bp after digestion with PstI restriction enzyme in accessions 49-49. Arrow indicates the undigested amplicon showing in accessions 45 (Jeera Sail-a) and 61 (Champaran Basmati).

also showed highly significant and positive association with BRLB (0.67) and BRL (0.59). However, it revealed negative correlation with RRB (-0.33). BRL showed highly significant positive association with BRLB (0.80). TGW showed highly significant positive correlation with BRL (0.79), RRL (0.77), BRB (0.67) and RRB (0.52).

Phenotypic Diversity in Qualitative Traits

The grain color (lemma-palea color) was recorded in 96 accessions of aromatic rices. Straw colored hull (lemma and palea) was present in 65.96% accessions followed by gold furrows on straw colored hull in 15.62% and black colored in 14.58% of accessions. However, two accessions each showed brown spots on straw and brown tawny hull color. Husk was removed and observations were recorded for caryopsis pericarp color (Figure 3b). White colored caryopsis pericarp was observed in 84 genotypes (87.50%), followed by brown colored (10.42%) and light brown colored (2.08%). On the basis of brown rice length to width ratio aromatic rice landraces were classified into three size categories namely slender (21.88%), medium (73.96%) and bold (4.17%). On the basis of brown rice length, the germplasm under study is categorized in to three groups *viz*., short (76.04%), medium (16.67%) and long grained (7.29%) accessions.

GS3 C/A Allele Genotyping

The SF28 primers generated a 136-bp fragment from second exonic region of the gene '*GS3*' in all aromatic rice landrace accessions (Figure 8). *PstI*, a restriction enzyme (hexacutter), was used to digest the PCR amplicons. *Pst*I recognised the restriction site CTGCAG and cleaved the amplicons into two fragments of 110 and 26 bp. The restriction site of *PstI* is reported to be present in short to medium grained rice cultivars. This represents the wild type allele, whereas the amplicon remains undigested by pstI enzyme in domesticated allele. Because these varieties contain a C to A SNP mutation, which results in elimination of the enzyme restriction site (CTGAAG). The rice landraces with an undigested fragment of 136 bp were scored as presence (+) of C/A SNP mutation and those with digested fragment of 110 and 26 bp size were scored as absence (-) of C/A SNP mutation. In panel of 96 aromatic rice accessions, only two landrace accessions, IC419046 (Jeera Sail-a) and IC569188 (Champaran Basmati) possessed (2.08%) the domesticated allele (Figure 8). Remaining landrace accessions had both fragment of size 136 and 110 bp after restriction digestion. These accessions were scored as heterozygous with respect to this allele.

Discussion

Grain dimension traits are important for rice quality and consumers' acceptability. We have analyzed seven quantitative and two qualitative traits in 92 aromatic rice landraces accessions and found high extent of variability

for these traits. The earlier studies from India and abroad also reported high variability for grain morphological traits (Karim *et al*., 2007; Islam *et al*., 2018; Prasad *et al*., 2020; Dixit *et al*., 2022). In a 208 indigenous aromatic rice germplasm study, Prasad *et al.* (2020) reported a dehusked grain length of 3.4 to 7.2 mm with a mean of 4.7 mm, whereas the grain breadth was 1.3 to 4.4 mm, with a mean value of 2.3 mm. The grain length/ breadth ratio was between 1.3 and 4.4 with a mean value of 2.3. They also observed that 41% of rice germplasm showed very low (<15 g) TGW. Jaiswal *et al.* (2007) reported high heritability (98.0%) for grain length, breadth and L/B ratio, and TGW (10.90 – 24.00 g with a mean of 16.40 g) in a set of 25 aromatic rice landrace accessions from eastern Uttar Pradesh that included accessions of Kalanamak, Juhi Bengal, Kanak Jeera and Dhania. However, Karim *et al.* (2007) recorded 5.90 to 30.72 g TGW with high heritability of 98.5% in 41 aromatic rices. The variation for grain length (3.7 to 6.8 mm), width (1.2 to 2.3 mm) and L/B ratio (1.82 to 3.25) was reported by Bisne and Sarawgi (2008) in 32 accessions of Badshahbhog landrace.

The earlier studies also reported high heritability (88.0–99%) for grain length, grain breadth, grain L:B ratio and TGW (Nirmaladevi *et al*. 2015; Roy *et al*., 2021) and support our results. Medium range of GCV and PCV values for grain length (11.19 and 11.20%), breadth (10.05 and 10.09%) and length/breadth ratio (16.81 and 16.83%) was reported by Nirmaladevi *et al*. (2015). In another study of 213 traditional rice varieties from Chhattisgarh, India, Sahu *et al*. (2017) reported moderate levels of GCV and PCV for the grain traits *viz.,* brown rice length (12.60 and 12.85%), brown rice width (14.78 and 16.44%) and brown rice L/B ratio (13.89 and 16.10%). They also observed high genetic advance (24.68–27.36%) for these traits. However, in our study a higher range of genetic advance (30.72–46.26%) observed for all the traits except TGW, which showed the highest GA (74.92%). The minor variations in the findings are due to difference in genetic makeup of landrace materials analyzed in these studies.

In the present study, grain length showed positive association with grain breadth (0.32), which is lower than the value of correlation (0.67) reported for these traits by Lakhar and Tanti (2017) in aromatic rice germplasm of Assam, India. Ngangkham *et al*. (2018) found highly significant and strong positive correlation of grain length with grain length/ breadth ratio (GBLR; 0.91) and with TGW (0.58). Grain length contributes more to TGW as compared to grain breadth, while grain breadth negatively affects the GBLR (-0.54). Dixit *et al*. (2022) also reported highly significant positive correlation of brown rice (dehusked rice) grain L/B ratio with grain length (0.88) and significantly negative association with grain breadth (-0.508) and corroborates our findings.

In rice, *GS*3 is the major gene located on chromosome 3 that plays a pivotal role in regulation of grain length (Fan *et al*., 2006). In the present study, allele 'A' found in two accessions (2.1%), while in 94 accessions (97.9%) the SNP was in heterozygous form after molecular analysis of C/A SNP in *GS*3 gene in 96 aromatic rice accessions. Fan *et al*. (2008) reported the presence of 'C' allele in 142 rice varieties having grain length of 6.4 to 8.8 mm, whereas 38 varieties possessed allele 'A' with long grains (8.8 to 10.7 mm). Similarly, Ngangkham *et al*. (2018) also studied allelic polymorphism in 89 rice genotypes with respect to C/A SNP in *exon* 2 of *GS3* gene. They reported allele 'A' in 37.07% of the genotypes. Rasheed *et al*. (2022) screened 17 rice genotypes for the presence of C/A SNP in *exon* 2 of *GS3* gene and reported that domesticated allele was present in 15 rice accessions.

Conclusion

The current study revealed that accessions of aromatic rice landraces from India's Indo-Gangetic Plain region have significant genetic variation for grain morphometric traits. The genotypic and phenotypic coefficients of variation for grain phenotypic traits in the rice crop were found to be comparable. It suggests that these traits are quite stable in nature. In our germplasm grain length contributed more to 1000-grain weight as compared to grain breadth. The polymorphism revealed for C/A SNP in grain size gene *GS*3, which could be utilized for genetic enhancement of grain size in aromatic rices.

Acknowledgment

First author is grateful to UGC for granting junior research fellowship during his Ph.D. study. Authors are thankful to Director, ICAR-National Bureau of Plant Genetic Resources, New Delhi for providing research facilities and National Gene Bank, ICAR-NBPGR, New Delhi for the seeds of aromatic rice accessions.

References

Bisne, R., & Sarawgi, A. K. (2008). Agro-morphological and quality characterization of Badshah Bhog group from aromatic rice germplasm of Chhattisgarh. Bangladesh *J. Agric. Res.,* **33(3):** 479–492.

Brar, D. S., & Khush, G. S. (2018). Wild relatives of rice: a valuable genetic resource for genomics and breeding research. In: Mondal, T. K. and Henry, R. J. (eds.) The Wild *Oryza* Genomes, Compendium of Plant Genomes, Springer, Cham. pp 1–25. *Doi:10.1007/978-3-319-71997-9_1*

Buttery, R. G., Ling, L. C., Juliano, B. O., & Turnbaugh, J. G. (1983). Cooked rice aroma and 2-acetyl-1-pyrroline. *J. Agric. Food Chem.,* **31(4):** 823–826.

Champagne, E. T., Bett, K. L., Vinyard, B. T., McClung, A. M., Barton, F. E., Moldenhauer, K., Linscombe, S., & McKenzie, K. (1999). Correlation between cooked rice texture and rapid visco analyses measurements. *Cereal Chem*., **76(5):** 764–771.

Dixit, D., Siddiqui, N., Bollinedi, H., Krishnan, G., Malik, A., Bhowmick, P. K., Ellur, R. K., Nagarajan, M., Vinod, K. K., & Singh, A. K. (2022). Assessment of agro-morphological traits,

grain physical and physico-chemical properties in the Indian aromatic rice (Oryza sativa) germplasm. *Indian J. Agric. Sci.,* **92(9):** 1157–1161.

Fan, C., Xing, Y., Mao, H., Lu, T., Han, B., Xu, C., Li, X., & Zhang, Q. (2006). GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theor. Appl. Genet.*, **112:** 1164–1171.

Fan, C., Yu, S., Wang, C., & Xing, Y. (2008). A causal C–A mutation in the second exon of GS3 highly associated with rice grain length and validated as a functional marker. Theoretical and Applied Genetics, **118:** 465–472.

Harlan, J. R., De Wet, J. M. J., & Price, E. G. (1973). Comparative evolution of cereals. Evolution, **7(2):** 311-325.

Hijmans, R. J., Guarino, L., Cruz, M., & Rojas, E. (2001). Computer tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. Plant Genetic Resources Newsletter, **127:** 15–19.

Islam, M. Z., Akter, N., Chakrabarty, T., Bhuiya, A., Siddique, M. A., & Khalequzzaman, M. (2018). Agro-morphological characterization and genetic diversity of similar named aromatic rice (Oryza sativa L.) landraces of Bangladesh. *Bangladesh Rice J.*, **22(1):** 45–56.

Jaiswal, H. K., Srivastava, A. K., & Dey, A. (2007). Variability and association studies in indigenous aromatic rice (*Oryza sativa* L.). Oryza, **44(4):** 351–353.

Johnson, H. W., Robinson, H. F., & Comstock, R. E. (1955). Estimates of genetic and environmental variability in soybeans. *J. Agron*, **47(7):** 314–318.

Juliano, B. O., & Villareal, C. P. (1993). Grain quality evaluation of world rices. International Rice Research Institute, Manilla, p. 205.

Karim, D., Sarkar, U., Siddique, M. N. A., Miah, M. K., & Hasnat, M. Z. (2007). Variability and genetic parameter analysis in aromatic rice. *Int. J. Sustain.*, **2(5):** 15–18.

Khush, G. S. (2000). Taxonomy and origin of rice. In: Singh, R. K., Singh, U. S., Khush, G. S. (eds.) Aromatic Rices, Oxford & IBH Publishing Co. Pvt. Ltd, New Delhi, pp. 5–13.

Lahkar, L., & Tanti, B. (2017). Study of morphological diversity of traditional aromatic rice landraces (Oryza sativa L.) collected from Assam, India. *Ann. Plant Sci.,* **6(12):** 1855–1861.

Li, Y., Fan, C., Xing, Y., Jiang, Y., Luo, L., Sun, L., Shao, D., Li, X., Xiao, J., & Zhang, Q. (2011). Natural variation in GS5 plays an important role in regulating grain size and yield in rice. *Nat. Genet.*, **43(12):** 1266–1269.

McKenzie, K. S., & Rutger, J. N. (1983). Genetic analysis of amylose content, alkali spreading score, and grain dimensions in rice. *Crop Sci.*, **23(2):** 306–313.

Murray, M. G., & Thompson, W. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.*, **8(19):** 4321–4326.

Ngangkham, U., Samantaray, S., Yadav, M. K., Kumar, A., Chidambaranathan, P., & Katara, J. L. (2018). Effect of multiple allelic combinations of genes on regulating grain size in rice. PLoS One, **13(1):** e0190684.

Nirmaladevi, G., Padmavathi, G., Kota, S., & Babu, V. R. (2015). Genetic variability, heritability and correlation coefficients of grain quality characters in rice (*Oryza sativa* L.). SABRAO *J. Breed. Genet.*, **47(4):** 424–433.

Prasad, G. S. V., Padmavathi, G., Suneetha, K., Madhav, M. S., & Muralidharan, K. (2020). Assessment of diversity of Indian aromatic rice germplasm collections for morphological, agronomical, quality traits and molecular characters to identify a core set for crop improvement. *CABI Agric. Biosci.,* **1(1):** 1–24.

Rasheed, H., Fiaz, S., Khan, M. A., Mehmood, S., Ullah, F., Saeed, S., Khan, S. U., Yaseen, T., Hussain, R. M., & Qayyum, A. (2022). Characterization of functional genes GS3 and GW2 and their effect on the grain size of various landraces of rice (*Oryza sativa*). *Mol. Biol. Rep.*, **49(6):** 5397–5403.

Robinson, H. F. (1966). Quantitative genetics in relation to breeding on centennial of Mendelism. *Indian J. Genet. Plant Breed.*, **26:** 171–187.

Roy, A., Hijam, L., & Roy, S. K. (2021). Genetic variability and character association studies for quality attributing traits in rice (*Oryza sativa* L.). *Electron. J. Plant Breed.*, **12(4):** 1201–1208.

Ruan, B., Shang, L., Zhang, B., Hu, J., Wang, Y., Lin, H., Zhang, A., Liu, C., Peng, Y., Zhu, L., Ren, D., Shen, L., Dong, G., Zhang, G., Zeng, D., Guo, L., Qian, Q. & Gao, Z. (2020). Natural variation in the promoter of TGW2 determines grain width and weight in rice. *New Phytol.*, **227(2):** 629–640.

Sahu, P. K., Sharma, D., Mondal, S., Kumar, V., Singh, S., Baghel, S., Tiwari, A., Vishwakarma, G., & Das, B. K. (2017). Genetic variability for grain quality traits in indigenous rice landraces of Chhattisgarh India. *J. Exp. Biol. Agric.*, **5(4):** 439–455.

Shao, G., Wei, X., Chen, M., Tang, S., Luo, J., Jiao, G., Xie, L., & Hu, P. (2012). Allelic variation for a candidate gene for GS7, responsible for grain shape in rice. *Theor. Appl. Genet.* **125:** 1303–1312.

Singh, R. K., Singh, U. S., Khush, G. S., Rohilla, R., Singh, J. P., Singh, G., & Shekhar, K. S. (2000). Small and medium grained aromatic rices of India. In: Singh, R. K., Singh, U. S., Khush, G. S. (eds) Aromatic Rices, Oxford & IBH Publishing Co. Pvt. Ltd, New Delhi, pp. 155–177.

Sivasubramaniam, S., & Madhavamenon, P. (1973). Genotypic and phenotypic variability in rice. *Madras Agric. J.*, **60:** 1093–1096.

Song, X. J., Huang, W., Shi, M., Zhu, M. Z., & Lin, H. X. (2007). A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nat. Genet.*, **39(5):** 623–630.

Takano-Kai, N., Jiang, H., Kubo, T., Sweeney, M., Matsumoto, T., Kanamori, H., Padhukasahasram, B., Bustamante, C., Yoshimura, A., Doi, K., & McCouch, S. (2009). Evolutionary history of GS3, a gene conferring grain length in rice. Genetics, **182(4):** 1323–1334.

Unnevehr, L. J., Duff, B., and Juliano, B. O. (1992) Consumer demand for rice grain quality. International Rice Research Institute, Manila, Philippines, and International Development Research Center, Ottawa.

Verma, D. K., & Srivastav, P. P. (2022). Extraction, identification and quantification methods of rice aroma compounds with emphasis on 2-acetyl-1-pyrroline (2-AP) and its relationship with rice quality: a comprehensive review. *Food Rev. Int.,* **38(2):** 111–162.

Wang, C., Chen, S., & Yu, S. (2011). Functional markers developed from multiple loci in GS3 for fine marker-assisted selection of grain length in rice. *Theor. Appl. Genet.*, **122:** 905–913.

Xu, J. Y., Xue, Q. Z., Luo, L. J., & Li, Z. K. (2002). Genetic dissection of grain weight and its related traits in rice (*Oryza sativa* L.). *Chin. J. Rice Sci.,* **16(1):** 6–10.

Zeng, D., Tian, Z., Rao, Y., Dong, G., Yang, Y., Huang, L., Leng, Y., Xu, J., Sun, C., Zhang, G., Hu, J., Zhu, L., Gao, Z., Hu, X., Guo, L.,

Xiong, G., Wang, Y., Li, J., & Qian, Q. (2017). Rational design of high-yield and superior-quality rice. *Nat. Plants*., **3(4):** 1-5.
Zhu, Y. J., Sun, Z. C., Niu, X. J., Ying, J. Z., Fan, Y. Y., Mou, T. M., Tang, S. Q., & Zhuang, J. Y. (2019). Dissection of three quantitative trait loci for grain size on the long arm of chromosome 10 in rice (*Oryza sativa* L.). Peer J, 7, e6966. *doi:10.7717/peerj.6966*.

Supplementary Table 1: ANOVA for seven quantitative grain traits studied in aromatic rice germplasm

Source	*D.F.*	*RRL*	*RRB*	*RRLB*	*TGW*	*BRL*	*BRB*	*BRLB*
Treatment (ignoring Blocks)	95	2.56 **	0.13 **	0.85 **	26.61 **	1.55 **	0.09 **	0.49 **
Treatment: Check	3	24.33 **	0.02 *	5.94 **	78.69 **	15.79 **	0.01 **	5.27 **
Treatment: Test vs. Check	1	24.65 **	0.37 **	13.09 **	70.27 **	7.07 **	0.86 **	8.54 **
Treatment: Test	91	1.59 **	0.13 **	0.55 **	22.41 **	1.02 **	0.08 **	0.25 **
Block (eliminating Treatments)	5	0.01 ns	0.004 ns	0.03 ns	4.51 ns	0.05 ns	0.00025 ns	0.02 ns
Residuals	15	0.03	0.01	0.03	2.55	0.08	0.00068	0.04

*, ** Significant at 0.05 and 0.001 level of probability; Abbreviations: ns: non-significant; RRL: Rough rice length, RRB: Rough rice breadth, RRLB: Rough rice length to breadth ratio, TGW: Thousand grain weight, BRL: Brown rice length BRB: Brown rice Breadth; BRB: Brown rice length to breadth ratio.

Supplementary Table 2: The Eigen vectors, proportion of variance, cumulative proportion of variance and Eigen values for principal components for seven grain traits

Parameter	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*	*PC7*
RRL	-0.4916	0.0432	-0.1279	-0.1110	-0.2099	0.8268	-0.0416
RRB	-0.1769	-0.5505	-0.5163	-0.5879	0.0326	-0.2252	0.0411
RRLB	-0.3190	0.4135	0.5061	-0.6522	0.0512	-0.2068	0.0188
TGW	-0.4394	-0.2514	0.1760	0.2495	0.8057	0.0156	-0.0322
BRL	-0.4954	0.0477	-0.0682	0.3133	-0.3043	-0.3090	0.6792
BRB	-0.2142	-0.5493	0.4990	0.1404	-0.4539	-0.1380	-0.3984
BRLB	-0.3753	0.3962	-0.4199	0.1912	-0.0666	-0.3313	-0.6125
Eigenvalues	3.8737	2.1490	0.5643	0.2136	0.1317	0.0617	0.0059
Proportion	0.5534	0.3070	0.0806	0.0305	0.0188	0.0088	0.008
Cumulative Proportion	0.5534	0.8604	0.9410	0.9715	0.9903	0.9991	1.000

Abbreviation: RRL: Rough rice length, RRB: Rough rice breadth, RRLB: Rough rice length to breadth ratio, TGW: Thousand grain weight, BRL: Brown rice length BRB: Brown rice Breadth; BRB: Brown rice length to breadth ratio

Supplementary Table 3: Pearson correlation coefficients values between 7 quantitative grain traits studied in 96 aromatic rice landraces accessions

	RRL	*RRB*	*RRLB*	*TGW*	*BRL*	*BRB*	*BRLB*
RRL	---	0.325 (0.0012)	0.613 (0.0000)	0.773 (0.0000)	0.938 (0.0000)	0.323 (0.0013)	0.762 (0.0000)
RRB		---	-0.333 (0.0009)	0.519 (0.0000)	0.267 (0.0086)	0.633 (0.0000)	-0.109 (0.2908)
RRLB			---	0.340 (0.0007)	0.593 (0.0000)	-0.102 (0.3237)	0.673 (0.0000)
TGW				---	0.795 (0.0000)	0.670 (0.0000)	0.386 (0.0001)
BRL					---	0.364 (0.0003)	0.796 (0.0000)
BRB						---	-0.261 (0.0103)
BRLB							---

Data in parentheses means the p-values.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2023.00036.2.12

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Characterization of Exotic Tomato Germplasm from ICAR-NBPGR Gene Bank

Suresh R. Yerasu[1*], Shailesh K. Tiwari[1], Chithra D. Pandey[2] and Sushil Pandey[2]

Abstract

The present study describes the characterization of 148 exotic tomato accessions from ICAR-NBPGR, New Delhi gene bank. The degree of variance for morphological features was high. Mean plant height of 72.36 cm, number of primary branches 2.7, days to 50% flowering 74.9, number of flower clusters per plant 5.91, number of flowers per cluster 3.91, number of locules per fruit 3.08, fruit weight 62.06 g, pericarp thickness 4.32 mm, fruit length 45.98 mm, fruit width 45.9 mm, total soluble solids 4.81° Brix and yield per plant 0.28 Kg were recorded. The first eight PCA extracted accounted for 89.23% of the total variation based on 12 phenotypic characters. A significant positive association was observed among the fruit characters. High PCV, GCV, heritability and GAM were recorded for most of the characters studied. The high variability especially for economically important fruit characters among the tomato germplasm like EC716696, EC715399, EC705451, EC705439, EC699717, EC695044, EC695038, EC695037, EC759285, EC759989 and EC759255 recorded higher TSS values and, germplasm like EC753226, EC716696, EC753220 and EC759989 recorded higher fruit yield per plant, makes this germplasm a potential resource for future tomato breeding programmes.

Keywords: Tomato, Diversity analysis, Genetic variation, Genetic advance, Correlation and Principal component analysis.

[1]ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India.

[2]ICAR- National Bureau of Plant Genetic Resources, New Delhi, Delhi, India.

***Author for correspondence:**

yerasusureshreddy@gmail.com

Received: 12/06/2022 **Revised:** 19/03/2023

Accepted: 20/03/2023

How to cite this article: Yerasu, S.R., Tiwari, S.K., Pandey, C.D., Pandey, S. (2023). Characterization of Exotic Tomato Germplasm from ICAR-NBPGR Gene Bank. *Indian J. Plant Genetic Resources*. 36(2), 301-308.
DOI: 10.5958/0976-1926.2023.00036.2.12

Introduction

Plant genetic resources (PGR) form the basis of any crop improvement programme. India has a strong base of tomato research with both public and private sector institutions involved in tomato breeding. PGR exchange and their utilization in different crop improvement programmes is a continuous process. In order to fulfill future demands brought in by the introduction of new diseases, climate change, and increased demands for food and nutritional security, there is an ongoing quest for different new PGR (De Jonge, 2009). In India, for the management of PGR, National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi is the nodal organization under the aegis of the Indian Council of Agricultural Research (ICAR), New Delhi. The plant quarantine (Regulation of Import) into India, order 2003 specifies how any researcher or user in India must introduce or import germplasm if they need access to seeds or planting materials from other nations (Tyagi *et al.*, 2021).

Tomato (*Solanum lycopersicum* L.) is a globally important vegetable crop and is consumed both fresh and in the form of different processed products. It is a rich source of vitamins (A and C), minerals and antioxidants. In India, along with potato and onion, tomato is an important vegetable crop in respect of nutrition, consumption and price fluctuations. The Andean region, which today includes parts of Chile, Bolivia, Ecuador, Colombia, and Peru, is the center of the origin of

tomato (Bai and Lindhout, 2007). Way back in 18th century, tomato was introduced in India and most of the present tomato introductions are bred varieties (Seshadri and Srivastava, 2002). Tomato was first among 25 different crops based on the number of requests for seed during 2014-19 received by ICAR-NBPGR, New Delhi (Tyagi *et al.*, 2021). In collaboration with other national organizations, ICAR-NBPGR also undertakes multiplication, characterization, evaluation, documentation and PGR conservation to promote sustainable use.

Understanding the genetic variation is the prerequisite for utilization of any PGR in improvement programmes. One of the intricate characteristics that can be ascribed to the numerous associated plant characteristics is yield. In addition to associations among different characters, the degree of heritability with genetic advancement is also crucial for crop development. Heritability and genetic advancement of different characters are the main considerations while making selections (Bhandari *et al.,* 2017). The main aim of the current study was to phenotypically characterize exotic germplasm for yield and related characters and to assess its breeding potential. This will facilitate understanding the potential of exotic tomato germplasm for proper utilization of the germplasm in future breeding programmes.

Materials and Method

The current study was carried out at ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India (25°10'N latitude and 82°52'E longitude).

Plant Materials and Data Collection

A set of 148 exotic tomato accessions from National Gene Bank, ICAR-NBPGR, New Delhi was used in the study. Among them, 104 accessions were introduced from the World Vegetable Center in Taiwan, 40 from Jordan, and 4 from the SAARC - Agriculture Centre at the BARC Complex and Farm Gate in Dhaka (Table 1). All the exotic tomato accessions belong to *S. lycopersicum* L. that is cultivated tomato. The study also included five ICAR-Indian Institute of Vegetable Research (IIVR) released varieties (Kashi Amrit, Kashi Anupam, Kashi Aman, Kashi Hemant and Kashi Vishesh) as checks. Data was recorded for 12 agri-horticultural characters, namely, plant height (cm), number of primary branches, days to 50% flowering, number of flower clusters per plant, number of flowers per cluster, number of locules per fruit, average fruit weight (g), yield per plant (g), pericarp thickness (mm), fruit length (cm), fruit width (cm) and total soluble solids (TSS) in ^{0}Brix. Days to 50% flowering was recorded when 50% of the plants started flowering from the date of planting. All other parameters were the averages from five plants or fruits.

Experimental Design

Augmented randomized complete block design (ARCBD) was the experimental design to grow the accessions in the field. Checks were replicated in each block and treatments (germplasm accessions) were not replicated. 148 accessions were grown in 4 blocks with 37 accessions in each block. As 25 days old seedlings were transplanted in the main field at 60 × 45 cm spacing between rows and between the plants. Standard cultivation practices recommended for tomato crop has been followed to raise a good crop (ICAR, 2009).

Statistical Analysis

The R package 'augmented RCBD' was used to estimate adjusted averages for all the characters under consideration for all the genotypes (Aravind *et al.*, 2018). Genetic variability parameters, frequency distribution, and descriptive statistics were also estimated using the same R package. The following formulas were used to determine phenotypic variance, genotypic variance, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h2) (Lush, 1940), and genetic advance (GA) (Johnson *et al.*, 1955):

$$\text{Genotypic variance } (\sqrt[2]{g} = \sqrt[2]{P} - \sqrt[2]{e})$$

Here,

$\sqrt[2]{P}$ = Phenotypic variance = Sum of squares of test treatments (genotypes)

$\sqrt[2]{e}$ = Error Variance = Sum of squares of residuals (error)

w

$$\text{Phenotypic Coefficient of Variation (PCV)} = \frac{\text{Phenotypic Variance}}{\sqrt{\text{mean}}} \times 100$$

$$\text{Genotypic Coefficient of Variation (GCV)} = \frac{\text{Genotypic Variance}}{\sqrt{\text{mean}}} \times 100$$

$$\text{Broad sense heritability } (h^2) = \frac{\sqrt[2]{g}}{\sqrt[2]{P}}$$

$$\text{Genetic Advance (GA)} = k \times \sqrt{g} \times \frac{h^2}{100}$$

where, k = selection intensity and σg= genotypic standard deviation

Correlation analysis was performed using the R package 'metan' (Olivoto and Lúcio2020) and PCA using the R packages 'FactoMineR' (Le *et al.*, 2008).

Results and Discussion

Genetic Properties

Mean, range, variance, coefficient of variance, heritability and genetic advance.

Proper estimation and reporting of the genetic diversity within the germplasm is the foremost important activity for better utilization of any germplasm (Islam *et al.*, 2004). Since morphological features offer a straightforward, simple and less expensive method of quantifying genetic variation, they have been employed to estimate genetic diversity

Table 1: List and source of the introduced tomato accessions in the study

S. No	Accession	Country
1	EC700930	Taiwan, Province of China
2	EC700931	Taiwan, Province of China
3	EC700933	Taiwan, Province of China
4	EC700936	Taiwan, Province of China
5	EC700938	Taiwan, Province of China
6	EC705436	Taiwan, Province of China
7	EC705437	Taiwan, Province of China
8	EC705438	Taiwan, Province of China
9	EC705439	Taiwan, Province of China
10	EC705440	Taiwan, Province of China
11	EC705442	Taiwan, Province of China
12	EC705443	Taiwan, Province of China
13	EC705444	Taiwan, Province of China
14	EC705445	Taiwan, Province of China
15	EC705446	Taiwan, Province of China
16	EC705447	Taiwan, Province of China
17	EC705449	Taiwan, Province of China
18	EC705450	Taiwan, Province of China
19	EC705451	Taiwan, Province of China
20	EC705452	Taiwan, Province of China
21	EC705453	Taiwan, Province of China
22	EC715376	Taiwan, Province of China
23	EC715377	Taiwan, Province of China
24	EC715380	Taiwan, Province of China
25	EC695037	Taiwan, Province of China
26	EC715382	Taiwan, Province of China
27	EC715383	Taiwan, Province of China
28	EC715384	Taiwan, Province of China
29	EC715385	Taiwan, Province of China
30	EC715386	Taiwan, Province of China
31	EC715387	Taiwan, Province of China
32	EC715388	Taiwan, Province of China
33	EC715389	Taiwan, Province of China
34	EC715391	Taiwan, Province of China
35	EC715393	Taiwan, Province of China
36	EC715394	Taiwan, Province of China
37	EC715396	Taiwan, Province of China
38	EC715397	Taiwan, Province of China
39	EC715398	Taiwan, Province of China
40	EC715399	Taiwan, Province of China
41	EC695036	Taiwan, Province of China
42	EC695037	Taiwan, Province of China
43	EC695038	Taiwan, Province of China
44	EC695039	Taiwan, Province of China
45	EC695040	Taiwan, Province of China
46	EC695041	Taiwan, Province of China
47	EC695042	Taiwan, Province of China
48	EC695043	Taiwan, Province of China
49	EC695044	Taiwan, Province of China
50	EC695045	Taiwan, Province of China
51	EC699710	Taiwan, Province of China
52	EC699714	Taiwan, Province of China
53	EC699715	Taiwan, Province of China
54	EC699716	Taiwan, Province of China
55	EC699717	Taiwan, Province of China
56	EC721954	Taiwan, Province of China
57	EC721955	Taiwan, Province of China
58	EC721957	Taiwan, Province of China
59	EC721958	Taiwan, Province of China
60	EC721959	Taiwan, Province of China
61	EC721961	Taiwan, Province of China
62	EC721963	Taiwan, Province of China
63	EC716696	Taiwan, Province of China
64	EC759989	Taiwan, Province of China
65	EC759991	Taiwan, Province of China
66	EC759992	Taiwan, Province of China
67	EC759993	Taiwan, Province of China
68	EC759997	Taiwan, Province of China
69	EC759998	Taiwan, Province of China
70	EC759999	Taiwan, Province of China
71	EC760002	Taiwan, Province of China
72	EC760003	Taiwan, Province of China
73	EC760004	Taiwan, Province of China
74	EC760005	Taiwan, Province of China
75	EC760006	Taiwan, Province of China
76	EC760007	Taiwan, Province of China
77	EC760008	Taiwan, Province of China
78	EC760009	Taiwan, Province of China
79	EC760011	Taiwan, Province of China
80	EC752609	Taiwan, Province of China
81	EC752610	Taiwan, Province of China
82	EC752612	Taiwan, Province of China
83	EC752613	Taiwan, Province of China
84	EC752614	Taiwan, Province of China
85	EC752615	Taiwan, Province of China
86	EC752616	Taiwan, Province of China
87	EC752617	Taiwan, Province of China
88	EC752618	Taiwan, Province of China
89	EC753216	Taiwan, Province of China
90	EC753218	Taiwan, Province of China
91	EC753219	Taiwan, Province of China
92	EC753220	Taiwan, Province of China
93	EC753221	Taiwan, Province of China
94	EC753223	Taiwan, Province of China

Table cont....

S. No	Accession	Country	S. No	Accession	Country
95	EC753224	Taiwan, Province of China	122	EC759262	Amman, Jordan
96	EC753225	Taiwan, Province of China	123	EC759263	Amman, Jordan
97	EC753226	Taiwan, Province of China	124	EC759264	Amman, Jordan
98	EC753227	Taiwan, Province of China	125	EC759265	Amman, Jordan
99	EC753228	Taiwan, Province of China	126	EC759266	Amman, Jordan
100	EC753230	Taiwan, Province of China	127	EC759267	Amman, Jordan
101	EC753231	Taiwan, Province of China	128	EC759268	Amman, Jordan
102	EC753232	Taiwan, Province of China	129	EC759269	Amman, Jordan
103	EC753233	Taiwan, Province of China	130	EC759270	Amman, Jordan
104	EC738047	Dhaka, Bangladesh	131	EC759271	Amman, Jordan
105	EC738050	Dhaka, Bangladesh	132	EC759272	Amman, Jordan
106	EC738054	Dhaka, Bangladesh	133	EC759273	Amman, Jordan
107	EC738055	Dhaka, Bangladesh	134	EC759274	Amman, Jordan
108	EC739326	Taiwan, Province of China	135	EC759275	Amman, Jordan
109	EC759243	Amman, Jordan	136	EC759276	Amman, Jordan
110	EC759244	Amman, Jordan	137	EC759277	Amman, Jordan
111	EC759246	Amman, Jordan	138	EC759278	Amman, Jordan
112	EC759247	Amman, Jordan	139	EC759279	Amman, Jordan
113	EC759248	Amman, Jordan	140	EC759280	Amman, Jordan
114	EC759250	Amman, Jordan	141	EC759281	Amman, Jordan
115	EC759251	Amman, Jordan	142	EC759282	Amman, Jordan
116	EC759252	Amman, Jordan	143	EC759283	Amman, Jordan
117	EC759254	Amman, Jordan	144	EC759284	Amman, Jordan
118	EC759255	Amman, Jordan	145	EC759285	Amman, Jordan
119	EC759258	Amman, Jordan	146	EC759286	Amman, Jordan
120	EC759259	Amman, Jordan	147	EC759287	Amman, Jordan
121	EC759261	Amman, Jordan	148	EC759288	Amman, Jordan

Source: for accessions from serial number 1 to 103 and 108 World Vegetable Centre, P.O.Box 42, Shanhua Tainan-74199; for accessions from serial number 104 to 107 SAARC, Agriculture Centre ,BARC Complex, Farm Gate Dhaka-1215; for accessions from serial number 109 to 148 225 5th Floor Office # 504 P O Box 830917, Amman.

and cultivar development (Fufa *et al.*, 2005). In the present study, a significant range of variance was seen among the tomato accessions for the twelve characters. ANOVA analysis showed that variance for all the characters was highly significant, indicating inherent genetic differences. All the characters show wide range (Table 2). The coefficient of variation (CV %) varied from 3.47 for number of days to 50% flowering to 17.96 for yield per plant. In the past many studies used molecular markers in tomatoes to study the diversity (Hu *et al.*, 2012; Cebolla-Cornejo *et al.*, 2013; Corrado *et al.,* 2013; Zhou *et al.,* 2015), but in this study we used only morphological characters for diversity study. Morphological characterization is not only simple, less expensive but also informative for breeding programs and also high levels of diversity based on morphological characters are associated with tomato lines that have a low level of genetic diversity with molecular markers (Mazzucato *et al.*, 2008; Cebolla-Cornejo *et al.*, 2013). Morphological characters have been utilized for diversity studies in the past in tomatoes (Henareh *et al.*, 2015; Bhattarai *et al.*, 2016).

For all the characters, phenotypic variance was higher than genotypic variance, indicating the environmental influence on all these characters (data not presented). Except for days to 50% flowering and TSS, the remaining characters found to have high PCV and GCV. The heritability in broad sense was higher for all the characters under study. A good measure of the progress that may be anticipated as a result of applying selection in a population is genetic advancement (GA). Heritability along with genetic advances, would provide a more accurate estimate of selection value (Johnson *et al.,* 1955). GA as a percent mean was recorded as high for all the characters except for days to 50% flowering (10.91). Days to 50% flowering recorded low GCV and PCV in the germplasm studied, indicating low variation for the

Table 2: Genetic variability parameters of the tomato germplasm

Trait	*Mean*	*Range*	*CV (%)*	*GCV*	*GCV category*	*PCV*	*PCV category*	*ECV*	*hBS (h^2) (%)*	*hBS category*	*GA*	*GAM (%)*	*GAM category*
PH	72.36	38.25–114.22	4.97	20.97	High	21.54	High	4.93	94.77	High	30.47	42.1	High
PB	2.74	0.91–8.04	13.17	37.24	High	39.52	High	13.21	88.83	High	1.99	72.42	High
DFF	74.9	58.48–83.84	3.47	6.09	Low	7.01	Low	3.47	75.44	High	8.17	10.91	Medium
NC	5.91	2.27–35.12	7.04	87.47	High	87.74	High	6.9	99.38	High	10.62	179.89	High
NFPC	3.91	1.72–7.96	6.09	31.3	High	31.88	High	6.06	96.38	High	2.48	63.39	High
LN	3.08	1.93–7.03	5.6	30.37	High	30.88	High	5.6	96.72	High	1.9	61.61	High
FW	62.06	8.99–158.75	6.05	50.73	High	51.12	High	6.27	98.49	High	64.46	103.87	High
PT	4.32	0.93–7.35	7.66	36.9	High	37.73	High	7.87	95.65	High	3.21	74.44	High
L	45.98	2.24–67.85	4.08	23.48	High	23.84	High	4.09	97.06	High	21.95	47.73	High
W	45.9	3.6–77.8	4.59	22.41	High	22.88	High	4.61	95.94	High	20.79	45.29	High
TSS	4.81	2.21–7.31	4.58	16.85	Medium	17.46	Medium	4.56	93.18	High	1.61	33.57	High
YPP	0.28	0.01–2.4	17.96		High	128.38	High	18.62	97.9	High	0.74	259.28	High

PH = plant height (cm), PB = number of primary branches, DFF = days to fifty percent flowering, NC = number of flower clusters per plant, NFPC = number of flowers per cluster, LN = number of locules per fruit, FW = average fruit weight (g), PT = pericarp thickness (mm), L = fruit length (mm), W = fruit width (mm), TSS = total soluble solids (TSS) in ^{O}Brix , YPP = yield per plant (Kg), CV% = Coefficient of variation and GAM = genetic advance as 5 % of mean

character. This gave rise to medium GA as a percent mean for the character even though it recorded high for heritability in a broad sense parameter. In all the remaining characters, both GCV and heritability in the broad sense were high leading to high GA as a percent mean (Table 2). The variation in genotypes for the characters under study may be due to genetic diversity and differences in adaptability (Hassan *et al.,* 2021). Variability and genetic diversity among the genotypes and higher heritability offer higher genetic gains in breeding programmes (Olakojo and Adetula 2014; Bhattarai *et al.,* 2016).

Frequency Distribution of Yield and TSS

Genotypes EC705450 (1380 g), EC759989 (1680 g), EC753220 (2112 g), EC716696 (2226 g) and EC753226 (2400 g) gave fruit yield of more than one kilogram per plant. TSS has special significance in breeding aspects of tomatoes for the processing industry. TSS in the germplasm ranged from 2.21 to 7.31 with around 39% the genotypes recording TSS greater than 5 ^{0}Brix. Eleven genotypes namely, EC759255 (6.00), EC759989 (6.01), EC759285 (6.1), EC695037 (6.21), EC695038 (6.21), EC695044 (6.21), EC699717 (6.21), EC705439 (6.24), EC705451 (6.24), EC715399 (6.41) and EC716696 (7.31) recorded ≥ 6.00 TSS. From the breeding point of view, TSS has been reported to have a negative correlation with fruit size and yield. There are few reports where TSS was improved without much effect on yield (Eshed and Zamir, 1995; Yousef and Juvik, 2001). Increased TSS increases the turnover of processed goods like paste and lowers the cost of tomato processing (Beckles, 2012; Reddy *et al.,* 2020).

Correlation

Correlation analyses is an important component of any breeding program as associated characters influence the selection and genetic gain of complex characters like yield. Pearson correlation coefficients were calculated to assess character correlations (Figure 1). Yield per plant has a significant positive correlation with number of flower clusters per plant (r = 0.39, p<0.001), number of primary branches (r = 0.33, p<0.001) and number of flowers per cluster (r = 0.28, p<0.001). Tiwari and Upadhyay (2011) reported a positive and highly significant association between fruit yield and number of branches per plant. Yield

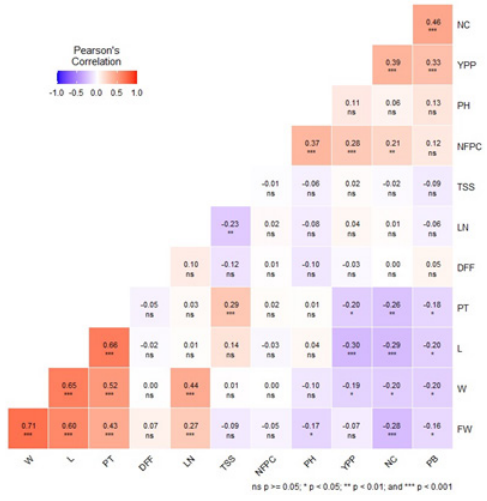

Figure 1: Graphical depiction of correlation among the quantitative characters in the tomato germplasm.

was negatively correlated with fruit length (r = -0.3, $p<0.001$), pericarp thickness (r=-0.2, $p<0.05$) and fruit width (r=-0.19, $p<0.05$). TSS was positively correlated with pericarp thickness (r = 0.29, $p<0.001$) and negatively correlated with number of locules per fruit (r = -0.23, $p<0.01$). The number of locules per fruit was positively correlated with fruit width (r = 0.44, $p<0.001$) and fruit weight (r = 0.27, $p<0.001$). Fruit characters ie fruit length, fruit width, fruit weight and pericarp thickness, were positively correlated among them and were negatively correlated with the number of primary branches and number of flower clusters per plant. Mishra and Nandi (2018) reported that fruit weight positively correlated with fruit width, length, number of locules per fruit and pericarp thickness. The number of flowers per cluster was positively correlated with plant height and number of flower clusters per plant. Similar observations were also reported by Kumar *et al.,* (2013) and Singh *et al.,* (2018).

Principal Component Analysis

Principal component analysis (PCA) was used to assess diversity on multivariate scales. The PCA simplifies the interrelationship among a large set of characters into a small

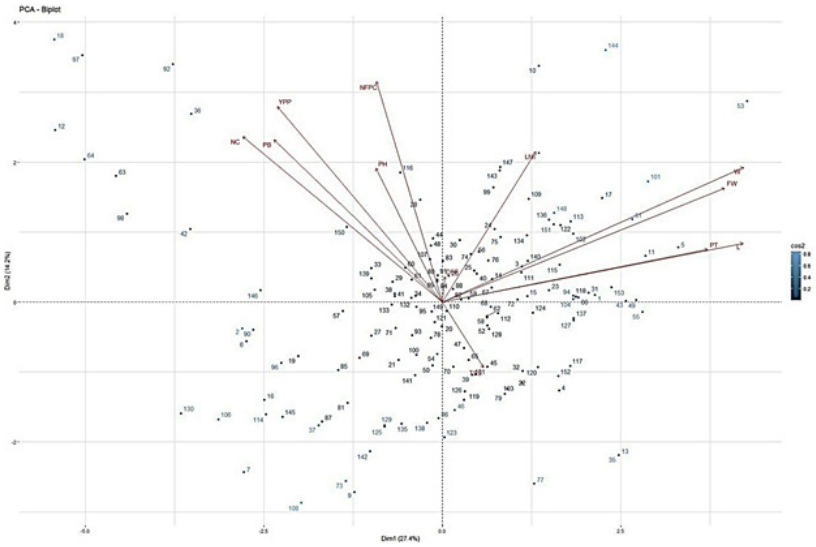

Figure 2: Two dimensional graphical representations of component patterns along with different variables based on PC1 and PC2

Table 3: Eigen values, proportion of the total variance represented by first eight principal components, cumulative percent variance and component loading of different characters in tomato

	PC1	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*	*PC7*	*PC8*
Eigen value	3.28	1.71	1.52	1.13	0.96	0.85	0.68	0.58
%variance Expressed	27.35	14.24	12.66	9.40	8.00	7.07	5.66	4.84
Cumulative variance Expressed	27.35	41.60	54.26	63.66	71.66	78.73	84.39	89.23
PH	-0.035	0.223	-0.033	0.935	-0.070	0.046	0.024	-0.024
PB	-0.065	-0.155	-0.104	0.213	0.091	0.835	0.235	-0.103
DFF	-0.007	0.024	-0.054	-0.064	0.990	0.021	-0.023	0.050
NC	-0.249	0.325	0.051	-0.160	-0.070	0.803	0.061	0.093
NFPC	0.022	0.910	-0.019	0.243	0.032	0.067	0.161	-0.008
LN	0.123	-0.006	-0.122	-0.017	0.056	-0.008	0.045	0.964
FW	0.827	-0.053	-0.229	-0.189	0.063	-0.164	0.192	0.136
PT	0.755	0.015	0.369	0.106	-0.018	-0.077	-0.144	-0.032
L	0.877	-0.007	0.081	0.080	-0.012	-0.088	-0.200	-0.107
W	0.813	0.042	-0.026	-0.099	-0.038	-0.072	-0.076	0.412
TSS	0.069	-0.016	0.952	-0.043	-0.055	-0.037	0.050	-0.118
YPP	-0.155	0.177	0.048	0.028	-0.030	0.240	0.911	0.042

PH = plant height (cm), PB = number of primary branches, DFF = days to fifty percent flowering, NC = number of flower clusters per plant, NFPC = number of flowers per cluster, LN = number of locules per fruit, FW = average fruit weight (g), PT = pericarp thickness (mm), L = fruit length, W = fruit width (mm), TSS = total soluble solids (TSS) in ^{0}Brix and YPP = yield per plant (Kg)

set of components without losing any essential information of the original data set. Thus PCA gives information of important characters that have a greater impact on the total variation and the degree of contribution (Sanni *et al.,* 2008). In the present study, the major portion of variance (63.65%) among the tomato genotypes is explained by the first four components with eigenvalue >1.0 (Table 3). The first component (PC1) accounted for 27.35% of variation largely through fruit characters like, average fruit weight, pericarp thickness, fruit length**,** fruit width; PC2 accounted for 14.24% of variation contributed through number of flowers per cluster, number of flowers clusters per plant and plant height, PC3 contributed for 12.66% of variation through total soluble solids and pericarp thickness and PC4 contributed for 9.4% of variation mainly through plant height, number of flowers per cluster and a number of primary branches. Two-dimensional graphical representations of component patterns along with different variables based on PC1 and PC2 is shown in Figure 2. The first two PCs contributed to 41.59% to the total variation with eigenvalues 3.28 and 1.71, respectively. These two PCs indicated that the main discriminatory characters were average fruit weight, fruit length, fruit width, number of flowers per cluster, number of flower clusters per plant, yield per plant, and number of primary branches. In similar studies, Mazzucato *et al*., (2008) and Hu *et al*., (2012) fruit characters contributed greatly to variation among tomato germplasm. In another study by Cebolla-Cornejo *et al.,* (2013) performed a diversity analysis of tomato germplasm, mainly including landraces from Spain. In their study, the first PC was also associated with fruit-size characters. Bhattarai *et al.,* (2016) studied the genetic diversity of 71 tomato genotypes based on its horticultural characteristics. In their study, five principal components explained more than 92% of the total phenotypic variation and fruit characters like fruit size, shape, and category were included in PCA1.

Conclusion

These diversified exotic germplasm are a reservoir of important genes/alleles for yield and fruit characters. Genotypes like EC753226, EC716696, EC753220 and EC759989 for yield per plant and, genotypes like EC716696, EC715399, EC705451, EC705439, EC699717, EC695044, EC695038, EC695037, EC759285, EC759989 and EC759255 for TSS can be used as principal foundation material in different breeding programmes for the development of new improved tomato varieties.

Acknowledgment

Authors are thankful to Director, ICAR-National Bureau of Plant Genetic Resources, New Delhi for funding under CRP-Agrobiodiversity through which ICAR–IIVR got germplasm for the study. The authors are also thankful to Director, ICAR- Indian Institute of Vegetable Research, Varanasi for facilitating field experiments.

References

Aravind, J., Sankar, M. S., Dhammaprakash, P. W.,&Kaur, V. (2018). augmentedRCBD: Analysis of Augmented Randomized Complete Block Designs. R package version 0.1.0, *https://aravind-j.github.io/augmentedRCBD/.*

Beckles, D. M., (2012). Factors affecting the postharvest sugars and total soluble solids in tomato (*Solanum lycopersicum* L.) fruits. *Postharvest Biol. Technol.* **63:** 129–140.

Bai, Y.,&Lindhout, P. (2007). Domestication and breeding of tomatoes: what have we gained and what can we gain in the future? *Ann. Bot.* **100(5):** 1085-94. *doi: 10.1093/aob/mcm150.*

Bhandari, H. R., Srivastava, K., & Eswar Reddy, G. (2017). Genetic variability, heritability and genetic advance for yield traits in tomato (*Solanum lycopersicum* L.). *Int J Curr Microbiol Appl Sci,* **6(7):** 4131–8.

Bhattarai, K., Louws, F. J., Williamson, J. D., & Panthee, D. R. (2016). Diversity analysis of tomato genotypes based on morphological traits with commercial breeding significance for fresh market production in eastern USA. *Aust. J. Crop Sci.* **10(8):** 1098–103.

Cebolla-Cornejo, J., Rosello, S., &Nuez, F. (2013). Phenotypic andgenetic diversity of Spanish tomato landraces. *Sci. Hortic.* **162:** 150-164.

Corrado, G., Piffanelli, P., Caramante, M., Coppola, M., & Rao, R. (2013). SNP genotyping reveals genetic diversity between cultivated landraces and contemporary varieties of tomato. *BMC Genom.* **14:** *DOI: 10.1186/1471-2164-14-835*.

DeJonge, (2009) Plants, Genes and Justice: An enquiry into fair and equitable benefit-sharing. Unpublished Ph D dissertation, Wageningen: Wageningen University. *http://www.fao.org/ agriculture/crops/thematic-sitemap/theme/seeds-pgr/conservation/en/)*.

Eshed, Y., & Zamir, D. (1995). An introgression line population of *Lycopersicon pennellii*in the cultivated tomato enables the identification and fine mapping of yield-associated QtI. *Genetics,* **141:** 1147-1162.

Fufa, H., Baenziger, P. S., Beecher, B. S., Dweikat, I., Graybosch, R. A., & Eskridge, K. M. (2005). Comparison of phenotypic and 1103 molecular marker-based classifications of hard red winter wheat cultivars. *Euphytica,* **145:** 133-146.

Hassan, Z., Ul-Allah, S., Khan, A. A., Shahzad, U., Khurshid, M., Bakhsh, A., *et al.* (2021). Phenotypic characterization of exotic tomato germplasm: An excellent breeding resource. *PLoS ONE,* **16(6):** e0253557

Henareh, M., Dursun, A., & Mandoulakani, B. A. (2015). Genetic diversity in tomato landraces collected from Turkey and Iran revealed by morphological characters. *Acta Sci. Pol.. Hortorum Cultus.* **14:** 87-96.

Hu, X. R., Wang, H., Chen, J., & Yang, W. C. (2012). Genetic diversity of Argentina tomato varieties revealed by morphological traits, simple sequence repeat, and single nucleotide polymorphism markers. *Pak. J. Bot.* **44:** 485-492.

ICAR, (2009). Handbook of Horticulture. New Delhi: Indian Council of Agricultural Research, pp. 464–470.

Islam, F. M. A., Beebe, S., Munoz, M., Tohme, J., Redden, R. J., & Basford, K. E. (2004). Using molecular markers to assess the effect of introgression on quantitative attributes of common bean in the Andean gene pool. *Theor. Appl. Genet.* **108:** 243-252.

Johnson, H. W., Robinson, H. F., & Comstock, R. E. (1955). Estimates of genetic and environmental variability in soybean. *J. Agron.* **47:** 314-318.

Kumar, D., Kumar, R., Kumar, S., Bhardwaj, M. L., Thakur, M. C., Kumar, R., Thakur, K. S., Dogra B. S., Vikram., A., Thakur.A., & Kumar, P. (2013). Genetic Variability, Correlation and Path Coefficient Analysis in Tomato. *Int. J. Veg. Sci.* **19(4):** 313-323.

Le, S., Josse, J., & Husson, F. (2008), FactoMineR: An R Package for Multivariate Analysis. *J. Stat. Softw.* **5(1):** 1-17.

Lush, J. L. (1940). Intra-sire correlations or regressions of offspring on dam as a method of estimating heritability of characteristics. *Proceeding of American Society of Animal Nutrition,* **1940:** 293–301.

Mazzucato, A., Papa, R., Bitocchi, E., Mosconi, P., Nanni, L., Negri, V., Picarella, M. E., Siligato, F., Soressi, G. P., Tiranti, B., & Veronesi, F. (2008). Genetic diversity, structure and marker-trait associations in a collection of Italian tomato (*Solanum lycopersicum* L.) landraces. *Theor. Appl. Genet.* **116:** 657-669.

Mishra, A., & Nandi, A. (2018). Correlation and path coefficient analysis for quality traits in tomato (*Solanum lycopersicon* L.). *J. pharmacogn. phytochem.* **7(1):** 1733–1738.

Olakojo, S. A., & Adetula, O. A. (2014). Comparison of qualitative and quantitative traits of some advanced breeding lines of tomato (*Lycopersicon esculentum* L.). *Afr. J. Plant Sci.* **8(10):** 457–61.

Olivoto, T., & Lúcio, A. D. (2020). metan: An Rpackage for multi-environment trial analysis. *Methods Ecol. Evol.* **11:** 783–789. *https:// doi.org/10.1111/2041-210X.13384*

Reddy, B. R., Singh, A. K., Pal, A. K., Reddy, Y. S., & Eswara Reddy, G. (2020). Combining ability and heterosis studies in tomato (*Solanum lycopersicum* L.) for quality traits and yield. *Int. J. Chem. Stud.* **8(2):** 2788-2792.

Sanni, K. A. I., Fawole, R. G., Guei, D. K., Ojo., & Somado, E. A. (2008). Geographical patterns of phenotypic diversity in *Oryza sativa* landraces of Côte d'Ivoire. *Euphytica,* **60:** 389-400.

Seshadri, V. S., & Srivastava, U. (2002). Genetic Resources and Improvements (Vegetable crops), p. 41–62. In: V.S. Seshadri and U. Srivastava (eds.). Evaluation of vegetable genetic resources with special reference to value addition. International conference on vegetables: Vegetables for sustainable food and nutritional security in the new millennium, organized by Dr. PremNath, Agricultural Science Foundation, Bangalore, Indian Society of Vegetable Science, New Delhi and Indian Institute of Horticultural Research, Bangalore, India.

Singh, A. K., Solankey, S. S., Akhtar, S., Kumari, P., & Chaurasiya, J. (2018). Correlation and Path Coefficient Analysis in Tomato (*Solanum lycopersicum* L.). *Int J Curr Microbiol Appl Sci,* **7:** 4278-4285.

Tiwari, J. K., & Upadhyay, D. (2011). Correlation and path-coefficient studies in tomato *Lycopersicon esculentum* Mill.). *Res. J. Agric. Sci.* **2(1):** 63–68.

Tyagi, V., Brahmi, P., Yadav, S. K., Pragya, Singh S. P., Singh, S., & Singh, K. (2021). Germplasm Access from ICAR-NBPGR and Use within India. *Indian J. Plant Genetic Resources,* **34(2):** 216–220.

Yousef, G. G., & Juvik, J. A. (2001). Evaluation of breeding utility of a chromosomal segment from *Lycopersicon chmielewskii* that enhances cultivated tomato soluble solids. *Theor. Appl. Genet.* **103:** 1022-1027.

Zhou, R., Wu, Z., Cao, X., & Jiang, F. L. (2015). Genetic diversity of cultivated and wild tomatoes revealed by morphological traits and SSR markers. *Genet. Mol. Res.* **14:** 13868-13879.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2023.00036.2.13

Indian Journal of Plant Genetic Resources

SHORT COMMUNICATION

Use of Fruit and Seed Characters for Identification of Edible *Artocarpus species* in India

Rinky R. Panda, Anjula Pandey*, Sandhya Gupta and Kailash C. Bhatt

Abstract

The genus *Artocarpus* (family Moraceae) consists of underutilized fruit crops. Taxa, *Artocarpus chama* Buch. -Ham. (Assam), *A. hirsutus* Lam. (mainly in Western Ghats, including Kerala, Karnataka), *A. heterophyllus* Lam. (Assam, Odisha and Bihar), *A. communis* J. R. Forster and G. Forster (Western Ghats, Andaman and Nicobar), *A. lacucha* Buch. -Ham. (Andhra Pradesh, Jharkhand, Odisha) and *A. gomeziana* Wall. (Assam, Andaman and Nicobar) are commonly known for edible value in different parts of India. Identification of taxa based on fruit and seed characters is of most significance in plant genetic resource conservation. Hence, the present study was on the characters of fruit and seed of edible *Artocarpus* species in India used for identification and developing taxonomic key. Since some of the species are not commonly distributed, additional data from floristic literature and e-floras has been used to validate records on distribution.

Keywords: *Artocarpus*, Distribution, India, Taxonomic key, Field identification, Crop wild relatives..

ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, Delhi, India

***Author for correspondence:**

anjula.pandey@icar.gov.in

Received: 28/07/2022 **Revised:** 29/10/2022

Accepted: 31/10/2022

How to cite this article: Panda, R.R., Pandey, A., Gupta, S., Bhatt, K.C. (2023). Use of Fruit and Seed Characters for Identification of Edible *Artocarpus species* in India. *Indian J. Plant Genetic Resources*. 36(2), 309-312. **DOI:** 10.5958/0976-1926.2023.00036.2.13

Introduction

The genus *Artocarpus* (family Moraceae) consists of taxa with evergreen and deciduous trees of underutilized fruit trees with a diversification centre in Southeast Asia. Jarrett (1959a, 1959b, 1960) refined *Artocarpus* after conducting extensive taxonomic research on local floras (Kochummen, 2000; Berg *et al*., 2006, 2011). There are nearly 70 species; 6 species have edible value, *Artocarpus altilis* Fosberg, *A. chama* Buch.-Ham., *A. hirsutus* Lam., *A. heterophyllus* Lam., *A. communis* J. R. Forster and G. Forster, *A. Lacucha* Buch.-Ham., *A. gomeziana* Wall. These taxa contain medicinally relevant secondary metabolites with biologically beneficial properties. In South-East Asia, Indonesia, the western portion of Java, and India, a variety of *Artocarpus* species are utilized as food and for traditional folk treatments.

Conservation of plant genetic resources management is essential for food security and agricultural biodiversity. Taxonomic identification must be ensured before the conservation of germplasm in a genebank. The endeavor of species identification and taxonomic classification has gone in era of molecular biology or ecology, where knowledge in this field of science is scarce (Ridley, 1986; Silva *et al*., 2011; Kirchoff *et al*., 2014).

The taxonomic keys generated in many species are insufficiently accessible to facilitate the identification of germplasm collections. They are not much used for identifying plant genetic resources as the germplasm is received in the form of seed or a part of the fruit. Taxonomic approaches based on fruit and seed traits are therefore essentially desired to accurately identify taxa, especially the underutilized species like *Artocarpus*.

Table 1: Distribution of *Artocarpus spp*. found in India

S. No.	*Name of species*	*Distribution in World*	*Distribution in India*	*Source*
1	*Artocarpus communis J. R. Forster & G. Forster* (syn-*Artocarpus altilis* (Parkinson) Fosberg; *A. incisus* (Thunberg) Linnaeus; *Radermachia incisa* (Thunberg)	Oceania from New Guinea through the Indo-Malayan Archipelago to western Micronesia	Western Ghats, Andaman and Nicobar Andhra Pradesh, Goa, Karnataka, Kerala, Lakshadweep	*https://www.cabi.org/isc/datasheet/1822*
2	*A. chama* Buch.-Ham. *(syn.- A. chaplasha* Roxb.*; A. melinoxylus* Gagnepain.*)*	Yunnan China, Bangladesh, Bhutan, India, Laos, Malaysia, Myanmar, Sikkim, Thailand	Hailakandi, Assam; Andaman and Nicobar	*https://indiabiodiversity.org*
3	*A. hirsutus* Lam. [(syn.- *Artocarpus pubescens* Willd., *Saccushirsutus* (Lam.) Kuntze]	Western Ghats, SW India, W Sri Lanka	Kerala, Karnataka, Maharastra, Tamilnadu, Goa	*https://en.wikipedia.org/wiki/Artocarpus_hirsutus https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:850390-1*
4	*A. heterophyllus* Lam.	India, South East of Indian Subcontinent, China to Philippines	Southern States, Assam, Odisha, Gujarat, Bihar, Central Himalayas, Andaman and Nicobar	*https://www.sciencedirect.com*
5	*A. lacucha* Buch.-Ham. (syn. *A. lakoocha* Roxb., *A. dadah* Miq., *A. ficifolius* W.T.Wang, *A. dasyphyllus* Miq.)	Indian Subcontinent and SE Asia	Andhra Pradesh, Jharkhand, Odisha, Bombay, Assam, West Bengal, Andaman and Nicobar	Flora of Peninsular India, Dagar and Singh (1999)
6	*A. gomeziana* Wall.	Assam to Western Malesia	Lakhimpur, Assam, Andaman and Nicobar	Kanjilal and Das (1984) Flora of Assam.

Though, by visual observation of the *Artocarpus* tree during blooming as well as in the vegetative stage, species are well distinguishable on the basis of their morphology. When germplasm is received as part in the form of fruit or seed, it is always mandatory to confirm the identity. In this paper, six edible *Artocarpus* species in India have been studied and the key characteristics of fruit and seed resulted in the development of a field key.

Data was collected on the distribution of different *Artocarpus* species present worldwide and in India (Table 1 and 2). Nearly 20 wild and cultivated species are found in India, i.e., *Artocarpus forbesii* King., *A. bracteatus* King*., A. calophylla* Kurz*., A. kunstleri* King*., A. integrifolia* L.fil., *A. peduncularis* Kurz, *A. lowii* King*, A. maingayi* King*, A. scortechinii* King*, A. nobilis* Thwaites*, A. lanceaefolia* Roxb*., A. chama* Buch.-Ham*., A. lacucha* Buch.-Ham*., A. gomeziana* Wall*., A. denisoniana* King (Hooker, 1875), *A. nitidus* Trécul (singh *et al*., 2017), *A. odoratissimus* Blanco.(Gupta and Chivers, 1999), *A. hirsutus* Lam. (Cokke, 1901), *A. communis* J. R. Forster and G. Forster and *A. heterophyllus* Lam. (Matthew, 1991). Among them 6 commonly occurring species of this genus have edible significance out of roughly 70 species worldwide present *viz. A. chama* Buch.-Ham., *A. hirsutus* Lam., *A. heterophyllus* Lam., *A. communis* J. R. Forster and G. Forster, *A. lacucha* Buchanan-Hamilton, *A. gomezianus* Wall.

Information on the distribution of *Artocarpus* spp. was collected from floras and e-flora of different states of India and China, along with other digital sources. Characters of fruits and seeds were recorded from the flora of Assam, West Bengal, Andhra Pradesh, Bombay, Tamil nadu, Goa, Diu, Daman, Dadar and Nagar Haveli, Meghalaya, British India, Andaman and Nicobar and flora are critically analyzed for identifying characters of *Artocarpus* species. To represent all the data gathered from various flora, data on the fruit and seed characteristics of various Indian *Artocarpus* species were retrieved. Analysis of key characteristics of fruit and seed of these species was done to develop taxonomic keys.

Distribution

Information collected on the distribution of the above 6 species found in India is presented in Table 1.

Seed and Fruit Characters

The evidence collected on fruit and seed characters of edible *Artocarpus* spp. found in India in different flora are as follows:

Taxonomic key

The key taxonomic characters were recorded as observed and validated from the literature. Classifying fruit and seed characters have been pulled to prepare the taxonomic key as follows

Key to species in genus Artocarpus

- *Fruit smooth*

Fruit globose-sub lobed or wrinkled.

Table 2: Distribution information of *Artocarpus spp.* found in India

Floras	*Hooker (1875)*	*Kanjilal and Das (1984)*	*Sanyal (1994)*	*Cokke (1901)*	*Matthew (1991)*	*Pullaiah and Moulali (1997)*	*Haridassan and Rao (1987)*	*Rao (1986)*	*Dagar and Singh (1999)*
A. communis J.R. Forster & G. Forster					✓				✓
A. chama Buchanan-Hamilton	✓	✓					✓		✓
A. hirsuta Lam.	✓			✓				✓	
A. heterophyllus Lam.	✓	✓	✓	✓	✓	✓	✓	✓	✓
A. lacucha Buch-Ham	✓	✓	✓	✓		✓			✓
A. gomeziana Wall.	✓	✓					✓	✓	✓

Fruit 5 to 7 cm. diameter, yellow when ripe, reddish brown on drying; seed oblong, broad, 1.5 cm diameter. ----------*Artocarpus lacucha*

Fruit unevenly globose-ovoid; 1–3 cm.---------- *Artocarpus gomezianus*

- *Fruit not smooth*
- *Fruit tuberculate*
- *Fruit non-cauliflorous, tubercle soft*

Fruit globose, 5 to 6cm dia, rust-colored brown on maturity; flattish rarely acute tips of the anthocarps; seed oblong- ovoid, size 1 to 2 cm.---------- *Artocarpus chama*

Fruit oblong, ellipsoid to conic-obovoid-globose, 15–30 × 8–15 cm, perianth not fleshy green to yellow, brown to black when mature; pericarp soft; mesocarp-milky white, fleshy calyx; seed oblong-cylindric ----------*Artocarpus communis*

- *Fruit cauliflorous, stiff tubercles*

Hexagonal tubercles, large, oblong, 1 to 1.5 ft. long, oblong-globose-ellipsoid - irregularly shaped; pale yellow when young, yellowish-brown when mature; seed size of a nutmeg, narrowly elliptic-oblong-reniform, oily 2.5–4 × 2–2.5 cm ----------*Artocarpus heterophyllus*

Fruit Spinous

Fruit ovoid, erect, size of a lemon, spines straight cylindric hispid, 0.63 cm perforate;

Seed ovoid, 1 to 2 cm. ---------- *Artocarpus hirsuta*

Keys for identification of *Artocarpus* species in the literature are cumbersome as they are based on characters of leaf, flowers, etc. They are difficult to consult when germplasm received in genebank is the form of fruit, part of fruit or seed. The present work attemptsto emphasize on correct identification of germplasm accessions prior to conserving them in gene bank using fruit and seed characters of different *Artocarpus* species of India. The taxonomic key developed here is highly beneficial for the identification of *Artocarpus* species.

Acknowledgment

The authors are gratefully acknowledging the facilities provided by Director, ICAR-NBPGR. This research paper is part of the M.Sc. (PGR), IARI of RRP.

References

Berg, C. C., Corner, E. J. H., & Jarrett, F. M. (2006). Flora Malesiana. Series I, Seed plants, Moraceae-genera other than Ficus, **17(1):**

Berg, C. C., Pattharahirantricin, N., Chantarasuwan, B., Santisuk, T., & Larsen, K. (2011). Flora of Thailand. Cecropiaceae & Moraceae. Bangkok: Forest Herbarium, Royal Forest Department, **10(4):**

Cooke, T. (1901). Flora of Bombay, Government of India, **3:** 157-159.

Dagar, J.C., and Singh, N.T. (1999). Plant Resources of the Andaman & Nicobar Islands. Bishen Singh Mahendra Pal Singh, 109-112.

Gupta, A., and Chivers, D.J. (1999). Asian primate communities. *Primate Communities*, **38.**

Haridassan, K., and Rao, R.R. (1987). Flora of Meghalaya, Bishen Singh Mahendra Pal Singh, India, *II*, 811-814.

Hooker, J.D. (1875). Flora of British India. 23A, New Connaught Place, Dehradun, **5:** 477-544.

Jarrett, F.M. (1959a). Studies in Artocarpus and allied genera, I. General Considerations. J. Arnold Arbor, **40:** 1–29.

Jarrett, F.M. (1959b). Studies in Artocarpus and allied genera, III. A revision of Artocarpus subgenus Artocarpus. J. Arnold Arbor, **40:** 113-155, 298-368.

Jarrett, F.M. (1960). Studies in Artocarpus and allied genera, IV. A revision of Artocarpus subgenus Pseudojaca. J. Arnold Arbor, **41:** 73–109, 111–140.

Kanjilal, U.N., and Das, A. (1984). Flora of Assam. Omsons Publications, *I*, 266-269.

Kirchoff, B.K., Delaney, P.F., Horton, M., and Dellinger-Johnston, R. (2014). Optimizing learning of scientific category knowledge in the classroom: The case of Plant Identification. CBE-Life

Sciences Education, ***13*:** 425-436.
Kochummen, K.M. (2000). Artocarpus. In: Soepadmo E. & Saw, L.G. (eds) Tree Flora of Sabah and Sarawak, Kuala Lumpur: Forest Research Institute Malaysia, Sabah Forestry Department & Sawarak Forestry Department, **3:** 187–212.
Matthew, K.M. (1991). An Exursion Flora of Cenral Tamilnadu, India, Oxford & IBH Publishing Company, 479-481.
Pullaiah, T., and Moulali, D.A. (1997). Flora of Andhra Pradesh, *Sci. Rep.* **2:** 894-895.
Rao, R.S. (1986). Flora of Goa, Diu, Daman, Dadra and Nagarhaveli. Flora of India Series 2. Botanical survey of India, **2:** 405-406.
Ridley, M. (1986). Evolution and Classification. Longman, London and New York.
Sanyal, M.N. (1994). Flora of Bankura District West Bengal, Bishen Singh Mahendra Pal Singh, 406-407.
Silva, H., Pinho, R., Lopes, L., Nogueira, A.J.A., and Silveira, P. (2011). Illustrated Plant Identification Keys: An Interactive tool to learn Botany. Comput. Educ., **56:** 969-973.
Singh, M.K., Sharma, C.L., Sharma, M. (2017). Comparative wood anatomy of four Artocarpus species of north east India with reference to their identification. In Wood is Good. Springer, Singapore, 73-81.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2023.00036.2.14

Indian Journal of Plant Genetic Resources

SHORT COMMUNICATION

Transgressive Segregation for Seed Protein in an Inter-specific *Cajanus* Cross

K.B. Saxena, Namita Srivastava* and R.V. Kumar

Abstract

Three inter-specific *Cajanus* crosses were assessed for their intra-population variation for protein content. In the unselected F_7 bulk population of a cross involving a wild species (*Cajanus scarabaeoides*) and an early maturing cultivar, "Baigani," the transgressive segregation for both high as well as low protein contents was observed. The high protein (35.6%) transgressive segregant produced 24% more protein than the wild species donor parent (28.7%). Similarly, the low-protein (18.8%) transgressive segregant had 17% less protein than the low-protein parent (22.7%). It is concluded that the parents of this cross carried different sets of dominant and recessive protein-controlling alleles; their complementary and/or additive gene effects produced these transgressive segregants.

Keywords: *Cajanus scarabaeoides, Cajanus cajan,* High protein, Genetic control, Segregation.

International Crops Research Institute for the Semi-arid Tropics, (ICRISAT), Patancheru, Telangana, India.

***Author for correspondence:**

namitasrivas@gmail.com

Received: 05/06/2022 **Revised:** 15/12/2022

Accepted: 19/12/2022

How to cite this article: Saxena, K.B., Srivastava, N., Kumar, R.V. (2023). Transgressive Segregation for Seed Protein in an Inter-specific *Cajanus* Cross. *Indian J. Plant Genetic Resources*. 36(2), 313-316.

DOI: 10.5958/0976-1926.2023.00036.2.14

Introduction

Among the rainfed legume crops pigeon pea occupies a high place due to its adaptation to subsistence agriculture, diverse utility applications, high protein (20–22%), and its role in combating malnutrition in different tropical and sub-tropical countries (Saxena *et al.* 2021). It is, therefore, believed that breeding high-protein pigeon pea cultivars may help further enhance protein availability. In the past, the lack of suitable high-protein pigeon pea donors restricted the breeders from taking any protein enhancement programme. The identification of certain *Cajanus* wild species as potent high-protein donors (Reddy *et al.*,1997) has regenerated interest in breeding high-yielding high, protein pigeon pea cultivars. In an inter-specific cross involving a pigeon pea [*Cajanus cajan* (L.) Mill sp.] cultivar and its wild relative [*C. scarabaeoides*] events of transgressive segregation were observed. In such genetic events a hybrid population produces individual(s) with extreme values outside the range of the parents. This paper reports the selection of transgressive segregants for seed protein within an F_7 bulk population for both high as well as low protein contents.

Materials and Methods

The Parents

A high-protein wild relative of pigeonpea (*Cajanus scarabaeoides*) was selected for this research. This species is a creeper (Figure 1) and has several undesirable agronomic

Figure 1: Plants of *C. scarabaeoides* (source: ICRISAT photo lab)

traits, such as photosensitivity, excessive biomass, and pod shattering. Flowering in this species is profuse but seed yield is low. The pods of *C. scarabaeoides* have prominent dense hairs with 3 to 5 ovules in each. Its seeds are small (2–3 g/100 seeds), dark brown and unfit for consumption but rich (28.7%) in protein content. The seed coat is hard and takes over three weeks to germinate. To generate inter-specific breeding populations this species was crossed with three early maturing pigeon pea cultivars (Baigani, T 21, and Pant A2), known for their good seed traits, adaptation, and yield.

Field Plot Technique

A basal doze of fertilizer @100 kg/ha DAP was applied and for good drainage, ridges 75 cm apart, were constructed in field along the slope. The seeds were manually placed at 2 to 3 cm depth with an inter-hills distance of 30 cm. The experimental area was irrigated for uniform germination and further irrigations were given as and when required. From flowering to maturity, the crop was monitored by plant protection team and sprayed with chemical insecticides to control pod borers.

Development of Breeding Populations

Due to hard seed coat, the seeds of *C. scarabaeoides* were scarified with a sharp blade to enable them to germinate rapidly. All the four genotypes were sown in a crossing block. In all the crosses, the wild species was used as male parent. The floral buds of pigeon pea cultivars were emasculated and pollinated simultaneously using fresh pollen collected from the wild species. In each flower bunch, only two buds were used in crosses. In each cross, most of the pollinated buds dropped and the 8 to 11% hybridization success was achieved. All the hybrid seeds were sown in pots, and kept inside a glasshouse. The matured pods were handpicked from each plant, and the plants were left to produce a second flush of flowers to fetch more F_2 seeds. All the F_2 plants were harvested at maturity, and their seeds bulked for generation advance. All the subsequent generations were raised in vertisols field. Since pigeon pea flowers are cross-pollinated by bees, mainly *Megachile* and *Apis* species, the breeding materials were grown each year under nylon field nets fixed on aluminum frames. The breeding materials were advanced through bulk method of breeding up to F_5 generation. A handful of pods were harvested from each plant and threshed separately. Subsequently, 20 seeds from each plant were bulked for generation advance. In F_6 generation, all the bulks were grown and F_7 single plants were harvested for protein determination and selection.

Protein Estimations

The fresh seeds harvested from single plants were cleaned and oven-dried at 55°C for 24 hours. An electronic seed counter was used to draw the samples of 100 seeds. The testa of each seed was removed using a tangential abrasive de-hulling device (TADD) and grinding of the decorticated grains was carried out using Udy cyclone mill. The nitrogen estimations of the samples were done with the help of Technicon Auto Analyzer and the protein values were determined by multiplying the nitrogen readings by a factor 6.25 (Singh and Jambunathan1982). For each plant two determinations were done, and their mean values were used for the study.

Results and Discussion

In the F_7 bulk of cross Baigani x *C. scarabaeoides,* a total of 1231 plants were assessed for their protein content. In cross T. 21 x *C. scarabaeoides* and Pant A 2 x *C. scarabaeoides* respectively, 219 and 268 plants were involved (Table 1). The results showed a wide range for protein content in all three crosses. In crosses T. 21 x *C. scarabaeoides* and Pant A 2 x *C. scarabaeoides,* the parental type segregants were recovered and the promising segregants were marginally (5–6%) better than the high protein donor *C. scarabaeoides.*

The cross Baigani x *C. scarabaeoides*, appeared best with protein content ranging between 18.8 to 35.6%. This range extended the parental values by significant margins in both directions. These observations indicated the emergence of transgressive segregants for protein content in this population. The highest protein value recorded

Table 1: Transgressive segregation for seed protein (%) in three inter-specific crosses

Cross	Cultivar	Wild species	Mid-parent	Protein range	Transgressive segregants		
					ID	%Protein	%Gain
Baigani x *C. scarabaeoides*	22.7	28.7	25.70	18.8–35.6	B-1	35.6	24.04
T. 21 x *C. scarabaeoides*	20.4	28.7	24.55	19.9–30.3	T-1	30.3	5.57
Pant A 2 x *C. scarabaeoides*	21.7	28.7	25.20	22.2–30.4	P-1	30.4	5.92

in a segregant was 35.6%. This unique recombinant had 24.04% greater protein than the wild species donor (28.7%). Similarly, at the other end, the segregant with the lowest (18.8%) protein had 17.18% less protein than the low-protein parent Baigani (22.7%). When the individual(s) in a segregating hybrid population exhibit extreme values outside the parental range, the situation is described as "transgressive segregation". Such segregants are produced when the alleles of a given trait in the two parents are different. They combine to produce a unique recombinant using their complementary and/or additive effects. In the other two crosses, where the same high protein donor was used, such transgressive segregants were absent; but some recombinants with slightly greater protein content than the donor were recovered (Table 1). This indicated that in comparison to CV Baigani, the genetic constitution of cvs. T.21 and Pant A2 was different as far as the genes controlling protein content is concerned.

Saxena *et al.* (communicated, unpublished) tested the progeny of this genotype and a control at three locations. They reported significantly large gains in protein over the control. This selection recorded, respectively 32.1, 32.2, and 32.3% protein at Gulbarga (17.4°N), Jalna (19.8°N), and Gwalior (26.2°N). The control values ranged between 22 and 23%. These data indicated significant superiority and high stability this high protein selection.

Dahiya *et al*. (1977), while studying the genetics of high-protein trait in pigeon pea reported the presence of 3 to 4 genes. Reddy and Singh (1981) further revealed that high protein genes are dominant in nature. Durga (1989) concluded from her studies that these genes are additive and/or complementary in nature. Rick and Smith (1953), Grant (1975), and Vega and Frey (1980) reported that the transgressive segregation or generation of extreme variability in a population is a consequence of complementary gene actions and product-wise, such events could appear on positive, negative, or both the sides of the performance-curve. The emergence of transgressive segregants is generally correlated with the genetic divergence of the parental lines and the presence of certain recessive complementary alleles whose expression is masked by the major/dominant alleles. These conclusions were further confirmed through marker-based QTL studies (De Vicente and Tanksley 1993). In the present case both low and high-protein transgressive segregants were recovered. Thus, it can be postulated that complementation of high-protein alleles from the wild species and CV. Baigani together produced the transgressive segregants. To understand the true genetic nature of protein content in pigeon pea some targeted genetic and molecular studies are needed.

Saxena and Sawargaonkar (2015) reported that this protein trait is highly stable across locations and years. Also, Singh *et al.* (1990) revealed that the key nutritional efficiency parameters such as true protein digestibility, biological value, and net protein utilization of the high protein selections, were similar to the control cultivar. Hence, their cumulative utilizable proteins and sulfur-containing amino acids are considerably greater than the commonly grown pigeon pea cultivars. Therefore, based on the overall assessment, the high-protein transgressive selections were nutritionally superior to the present cultivars. Such high-protein genetic stocks are valuable to pigeon peas' genetic wealth. Hence irrespective of their agronomic traits, they should be preserved for future protein-related genetic studies or breeding programmes. Since these selections also carry a portion of the cultivated type of genome, they will not suffer from any hybridization incompatibility or linkage drag which are common issues in any inter-specific or inter-generic breeding programme.

Conclusion

Here, we report the selection of a high protein (35.6%) transgressive segregant from an inter-specific *Cajanus* cross. This is the highest protein value ever recorded in pigeon pea. Besides this, the protein quantity present in this line is nutritional efficient and stable across the environments. These traits make it a valuable germplasm resource for use in future pigeon pea breeding programmes.

Acknowledgments

The authors acknowledge Dr JM Green and Dr DG Faris, Principal Pigeonpea Breeders, ICRISAT, for their guidance and support in conducting this research.

References

Dahiya, D.S., Brar, J.S., & Bhullar B.S. (1977). Inheritance of protein content and its correlation with grain yield in pigeon pea (*Cajanus cajan* L. Millsp.). *Plant Foods Hum. Nutr.*, **27:** 327-334.

De Vicente, M.C., & Tanksley, S.D. (1993). QTL analysis of transgressive segregation in an inter-specific tomato cross. *Genetics*, **134:** 585-596.

Durga, B.K. (1989). Genetic studies on protein content and nitrogen accumulation in pigeonpea. Ph D. Thesis, Osmania University, Hyderabad, India.

Reddy, L.J., Saxena, K.B., Jain, K.C., Singh, U., Green, J.M., Sharma, D., Faris, D. G., Rao, A.N., Kumar, R.V. & Nene, Y.L. (1997). Registration of high protein pigeonpea elite germplasm ICPL 87162. *Crop Sci.*, **37:** 294.

Reddy, L.J., & Singh U. (1981). Improving protein quality in pigeonpea through the use of wild relatives. *International Pigeonpea Newsletter*, **1:** 22-23.

Rick, C.M., & Smith, P.G. (1953). Novel variation in tomato species hybrids. *Am. Nat.*, **87:** 359-375.

Saxena, K.B., Choudhary, A.K., Dalvi, V.A., et al. (2021). Pigeonpea is significantly more than just a delicious pulse. *J. AgriSearch*, **8:** 177-187.

Saxena, K.B., & Sawargaonkar, S.L. (2015). Genetic enhancement of seed proteins in pigeonpea – methodologies, accomplishments, and opportunities. *Int. J. Sci. Res.*, **4:** 3-7.

Saxena, K.B., Saxena, R.K., & Reddy, L.J. (2023). Breeding high protein pigeonpea genotypes and their agronomic and biological assessments. *Plant Breed.*, **142(2):** 129-139.

Singh, U., & Jambunathan, R. (1981). Methods for the estimation of protein in pigeonpea (*Cajanus cajan* L. Millsp.) and the relationship between whole grain and dhal protein contents. *J. Sci. Food Agric.*, **32:** 705-710.

Singh, U., Jambunathan, R., Saxena, K.B., & Subramaniam, N. (1990). Nutritional quality evaluation of newly developed high protein genotypes of pigeonpea (*Cajanus cajan* L. Millsp.). *J. Sci. Food Agric.*, **50:** 201-209.

Vega U. & Frey K.J. (1980). Transgressive segregation in inter- and intraspecific crosses of barley. *Euphytica*, **29:** 585-694.

P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.5958/0976-1926.2023.00036.2.15

Indian Journal of Plant Genetic Resources

BOOK REVIEW

Genus *Allium* L. of the Indian Region: A Field Guide for Germplasm Collection and Identification

Ravi Gowthami and Subhash Chander

Introduction

Allium L. is one of the largest monocotyledonous genus with more than 1,100 species known for food, flavor, medicinal and ornamental value. Indian gene centre has a rich diversity of cultivated and lesser-known wild *Allium* species with 35 to 40 taxa distributed in different eco-geographical regions of the country. Explorers face challenges in the correct identification of species during explorations. Hence, there is an utmost need of field keys for correct identification.

The book entitled genus *Allium* L. of the Indian Region: A Field Guide for Germplasm Collection and Identification" authored by Anjula Pandey, Pavan Kumar Malav, KM Rai and SP Ahlawat discusses in-depth procedures for the collection and identification of Indian *Alliums* and is comprised of six chapters. Chapter 1 furnishes an introduction to the genus *Allium* and its diversity globally and nationally with prospects and thrust areas of research. Chapter 2 highlights the methodology for the collection of germplasm, post-collection processing, identification tools and methods: consulting herbaria and online records, the study of micro-morphological characters for identification, and on-spot documentation by photography. The chapter is well concluded by providing important key points to be noted during germplasm collection and identification. Chapter 3 provides intense knowledge on different field aids for collection and identification from conventional to recent digital tools (digital field book, GIS maps, digital library). Chapters 4 and 5 provide a user-friendly taxonomic key to the genus *Allium* L. in India and important guidelines for recording the data during germplasm collection and for identification. Chapter 5 is nicely illustrated by a diagrammatic sketch of important characters used for recording. Chapter 6 presents the description of selected taxa and their taxonomy, synonyms, habitat, flowering period, distribution (world and India) and biological status, mode of propagation, and field notes separately with beautiful representative images based on the detailed work undertaken at ICAR-NBPGR over the years. The images and detailed description of individual species *viz*., *Allium ampeloprasum* L., *A. auriculatum* Kunth, *A. carolinianum* DC., *A. cepa* var. *aggregatum* G. Don, *A. cepa* var. *cepa L*., *A. chinense* G. Don, *A. consanguineum* Kunth, *A. fasciculatum* Rendle, *A. fistulosum* L., *A. hookeri* Thwaites, *A. humile* Kunth, *A. negianum* A. Pandey, K.M. Rai, P.K. Malav & S. Rajkumar, *A. oreoprasum* Schrenk, *A. przewalskianum* Regel, *A. roylei* Stearn, *A. sativum* L., *A. schoenoprasum L*., *A. stracheyi* Baker, *A. tuberosum* Rottler *ex Spreng*., *A. wallichii* Kunth, *etc*. will facilitate the explorers/collectors for correct identification of the material on-spot. The authors have also enlightened their readers with a list of different species of indigenous *Allium* germplasm conserved in the field gene bank at ICAR-NBPGR, RS Bhowali, the only field gene bank in the country with 25 native species/taxa. Photo plates of few exotic species *viz*., *A. altaicum* Pall, *A. ascalonicum* L., *A. ledebourianum Schult. & Schult. f., A. oschaninii O.* Fedtsch., *A. ramosum* L., *A. senescens* L., *and A. obliquum* L. maintained in this field genebank have also been provided in the book.

Tissue Culture and Cryopreservation Unit, Indian Council of Agricultural Research - National Bureau of Plant Genetic Resources, New Delhi, Delhi, India

***Author for correspondence:**

Gowthami.R@icar.gov.in

Received: 20/09/2022 **Revised:** 26/12/2022

Accepted: 05/01/2023

How to cite this article: Gowthami R and Chander S. (2023). Genus *Allium* L. of the Indian Region: A Field Guide for Germplasm Collection and Identification. *Indian J. Plant Genetic Resources*. 36(2), 317-318. **DOI:** 10.5958/0976-1926.2023.00036.2.15

The authors have also highlighted the future concerns of *Allium* genetic resources collection and conservation. The book has also provided a glimpse of future thrust areas for the benefit of researchers to plan their future research in the important areas concerning genus *Allium*.

To abridge, the authors have put in splendid efforts to bring out a handy, user-friendly field guide for collecting and identifying the genus *Allium* L. of Indian Region. The authors must be congratulated on bringing this field guide in an organized manner into public domain for the benefit of students, research scholars, taxonomists, ecologists and explorers. The key characteristics for identification supported with good photographs in this book are unique. We believe that this first-of-its-kind pictorial field guide would help researchers/collectors/botanists in the correct methodology for collecting and identifying *Allium* species on-spot in the field. Overall, this book is worthwhile reading for teachers/students/researchers/botanists/conservationists working on *Allium*. Finally, we take this opportunity to compliment the senior author of this book, Dr. Anjula Pandey, who immensely worked on the *Allium* genus for the past 9 years and associated young explorers in bringing out this book.

For more details: Pandey A, PK Malav, KM Rai and SP Ahlawat (eds) (2022) *Genus Allium L. of the Indian Region: A Field Guide for Germplasm Collection and Identification*. ICAR-National Bureau of Plant Genetic Resources, New Delhi, India, 109p + i-vi.

Indian J. Plant Genet. Resour. 36(2): 319-340 (2023)

Plant Germplasm Registration Notice*

The Plant Germplasm Registration Committee of ICAR in its XXXXIV[th] meeting held on June 30[th], 2021 at the ICAR-National Bureau of Plant Genetic Resources, New Delhi. A total of 43 proposals were received for registration. Out of that, 24 proposals were considered for registration. Finally, 21 applications covering nine 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. Introgression line IL 274 (IC0637547; INGR21091), a rice (*Oryza sativa* L.) germplasm for bacterial blight resistance.

Kuldeep Singh, Kumari Neelam*, Yogesh Vikal, Dharminder Bhatia, JS Lore and GS Mangat
Punjab Agricultural University, Ludhiana, Punjab-141004, India
**Email: kneelam@pau.edu*

Bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae*, is one of the major constraints of rice productivity in Southeast Asia (Mew 1987; Chen *et al*. 2011). Here, we want to register an introgression line IL 274, derived from *Oryza glaberrima* accession IRGC 102600B with novel bacterial blight resistance gene *xa-45*(t) (Neelam *et al*., 2020). The inheritance studies in F_2 and $F_{2:3}$ populations of a cross involving Pusa44 and IL274 revealed the presence of single recessive locus controlling resistance to the *Xanthomonas* pathotype seven. The QTL mapping identified a major locus on the long arm of rice chromosome 8 with a LOD score of 33.22 between the SNP markers C8.26737175 and C8.26818765. The peak marker, C8.26810477, explains 49.8% of the total phenotypic variance and was positioned at 202.90 cM on the linkage map. This major locus spans 80 kb region on Nipponbare reference genome IRGSP-1.0 and contains 9 candidate genes. A co-segregating STS marker was developed from the LOC_Os08g42410 for efficient transfer of this novel gene to elite cultivars.

Morpho-agronomic characteristics: The Agro-morphological features includes 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. Leaf auricles, pubescence of leaf blade surface and anthocyanin coloration of lemma apex are absent.

Associated characters and cultivation Practices: The genetic stock IL274 has bacterial blight resistance against all the ten prevalent *Xanthomonas* pathotypes of Punjab and Northern states of India. The genetic stock carries bacterial blight resistance gene *xa-45 (t)* from *Oryza glaberrimma* accession IRGC102600b on the long arm of chromosome 8 of rice.

References

Chen S, Liu X, Zeng, L, Ouyang D, Yang J, Zhu X (2011) Genetic analysis and molecular mapping of a novel recessive gene Theor. Appl. Genet. **122:** 1331.https://doi.org/10.1007/s00122-011-1534-7

Mew T W (1987). Current status of future prospects of research on bacterial blight of rice. *Annu. Rev. Phytopathol.* **25:** 359–382. https://doi.org/10.1146/annurev.py.25.090187.002043

Neelam, K., Mahajan, R., Gupta, V. *et al*. High-resolution genetic mapping of a novel bacterial blight resistance gene xa-45(t) identified from *Oryza glaberrima* and transferred to *Oryza sativa*. *Theor. Appl. Genet*. **133:** 689–705 (2020). *https://doi.org/10.1007/s00122-019-03501-2*.

Table 1: Frequency of resistant and susceptible plants in F_2 and $F_{2:3}$ populations and Chi squareanalysis

	Number of plants					
Population	*Resistant*	*Segregating*	*Susceptible*	*Total*	x_c^2	x_t^2
F_2	91	-	221	312	2.57	x^2 (0.05,1) = 3.84
$F_{2:3}$	74	158	80	312	0.27	x^2 (0.75, 2) = 0.57

Edited by: Anju Mahendru Singh and Anjali Kak, Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110 012.

Acknowledgement is due to Arup Das for providing technical assistance in compilation.

2. Minatik Charang (ARC 10075); IC0597237 (IC0597237; INGR21092), a rice (*Oryza sativa* L.), germplasm with high grain protein content (12–14%).

Torit Baran Bagchi*[1], Krishnendu Chattopadhyay[1], Bishnu Charan Marndi[1], Awadhesh Kumar[1], Lotan Kumar Bose[1], Padmini Swain[1], BC Patra[1], SG Sharma[2] and Ruchi Bansal[2]

[1]*ICAR-National Rice Research Institute, Cuttack, Odisha-753006, India*

[2]*ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012, India*

**Email: torit.crijaf09@gmail.com*

ARC 10075 with high grain protein content (13-16%) was identified from the genetic stock of the Assam Rice Collections (ARC) of the CRRI (NRRI) Rice Gene Bank. Three glutelin bands were highly expressed in the high-protein cultivar. They showed higher activity of nitrate reductase (NR) and glutamic dehydrogenase (GDH) at seedling stage (one-week-old) and maximum tillering stage (three week-old)(DARE/ICAR Annual Report 2011-12). It was found that this germplasm does contain an average 12.03% with a range of 10.43%- 15.27% grain protein as tested over the years and different environments (Table 1). Nitrate reductase (NR) and Nitrite reductase (NiR) activity was found to be higher for ARC 10075 as compared to low protein counterpart (ICAR-NRRI Annual Report 2019). It was also found with higher free amino acids (0.26%), methionine (0.082%), Lysine (0.049%) and Tryptophan (0.063%) (Mohanty *et al*. 2011). The fractions of different soluble protein in this germplasm are 0.434%, 1.415%, 0.443% and 12.864% of albumins, globulins, prolamins and glutelins, respectively (Chattopadhyay et. al. 2019a). The yield of this germplasm was 2.45 t/ha. The average maturity duration was found medium (130 days). It has tall (140cm) plant type and the grain type is medium slender.It is having high head rice recovery (61%), high alkali spreading value (7) and medium amylose content (24.6%) (ICAR-NRRI Annual Report 2013-14). A consistent QTL for grain protein content (*qGPC1.1*) and two QTLs for single grain protein content (*qSGPC2.1, qSGPC7.1*) were detected using backcross derived population from ARC 10075/ Naveen (Chattopadhyay *et al*. 2019b). The first high protein rice variety of India, CR Dhan 310 was developed using this donor, ARC 10075.

Justification

This valuable rice germplasm was found stable in high seed protein content (12%) over the years and environments. This donor was also utilized in development of first high protein rice variety of India, CR Dhan 310 (10% protein content).

Table 1: Evaluation of ARC 10075 for grain protein content under multi- environmental condition

Sl. No.	*Year of evaluation*	*Season*	*Grain protein content related information*	*References*
1	2008	Wet season	15.27% grain protein content in brownrice	DARE/ICAR Annual Report 2011-12; Mohanty *et al*. 2011
2	2010	Wet season	10.43% grain protein content in polished rice with 220.24g proteinyield/plant	Chattopadhyay *et al,*2011
3	2011	Wet season	11% grain protein content in polished rice	Chattopadhyay *et al.* 2018
4	2013	Wet season	12.8% protein content	ICAR-NRRI Annual Report, 2013-14
5	2014-15	Rabi season	13.00 % grain protein content and wasricher in nearly all amino acids as compared to check, Naveen.	ICAR-NRRI Annual Report, 2014-15, https://icar-nrri.in/wp- content/ uploads/2018/06 /2014-15.pdf
6	2014	Rabi season	10.80% in milled rice	Chattopadhyay *et al.* 2019a
7	2014	Wet season	10.88% in milled rice	Chattopadhyay *et al.* 2019a
8	2019	Wet season	Nitrate reductase (NR) and Nitrite reductase (NiR) activity was found to be higher for ARC 10075	ICAR-NRRI Annualreport 2019.
	Average		12.03% grain protein content over the environments	
	Range		10.43- 15.27%	

Moreover, consistent QTLs for grain protein contents were detected using this germplasm.

References

Chattopadhyay K, Das A and Das SP (2011) Grain protein content and genetic diversityof rice in north eastern India. *Oryza* **48(1):** 73-75.

Chattopadhyay K, Sharma SG, Bagchi TB, Molla K A, Sarkar S, Marndi BC, Sarkar A, Dash K and Singh ON (2018) Development of recombinant high yielding lines with improved protein content in rice (*Oryza sativa* L.) *J. Agric. Sci.* 1–17. *https://doi.org/10.1017/ S0021859618000230.*

Chattopadhyay K, Sharma SG, Bagchi TB, Mohanty B, Sardar SS, Sarkar S and Singh ON (2019a) High-protein rice in high-yielding background, cv. Naveen. *Curr. Sci.***117(10):** 1722-1726.

Chattopadhyay K, Behera L, Bagchi TB, Sardar SS, Moharana N, Patra NR, ChakrabortiM, Das A, Marndi BC, Sarkar A, Ngangkham U, Chakraborty K, Bose LK, Sarkar S, RayS, Sharma SG (2019b) Detection of stable QTLs for grain protein content in rice (*Oryza sativa* L.) employing high throughput phenotyping and genotyping platforms. *Sci. Rep.* **9(1):** 1-16.

DARE/ICAR Annual Report 2011-12

ICAR-NRRI Annual Report, 2013-14

ICAR-NRRI Annual Report, 2014-15, *https://icar-nrri.in/wp-content/uploads/2018/06/2014-15*

ICAR-NRRI Annual Report 2019.

Mohanty A, Marndi BC, Sharma SG and Das A (2011) Biochemical characterization oftwo high protein rice cultivars from Assam rice collections. *Oryza*. **48(2):** 171-174.

3. Phougak (D82) (IC0639794; INGR21093), a rice (*Oryza sativa* L.) germplasm for tolerance to sheath blight. Resistance to neck blast. resistance to leaf blast.

Jyothi Badri[1*], V Prakasam[1], RM Sundaram[1], MS Prasad[1], GS Laha[1], VP Bhadana[2], LV Subba Rao[1] and C Priyanka[1]

[1]*ICAR-Indian Institute of Rice Research, Hyderabad, Telangana-500030, India*

[2]*ICAR-Indian Institute of Agricultural Biotechnology, Ranchi, Jharkhand-834010, India*

[*]*Email: jyothirishik@gmail.com*

Rice is an important staple food for more than half of the global population. Rice production is constrained by several biotic and abiotic stresses and sheath blight (ShB) is one of the major devastating disease and responsible for 70% of yield losses and quality degradation. ShB is caused by necrotrophic soil born pathogen *Rhizoctonia solani* AG-1 IA which affects sheath, leaf and panicle. It produces water soaked brown color oval shaped lesions on sheath, irregular lesion on leaf and produces sclerotia during unfavourable conditions. So far, no immune sources to sheath blight have been identified throughout the world. Use of chemical fungicides for ShB disease management not only has an adverse impact on the ecology, environment but it also stresses the pathogen to develop virulentraces.

Landraces from north eastern part of the country along with wild introgression lines, tropical japonica accessions, mutants, elite cultivars totalling about 1500 were evaluated for sheath blight resistance in 2012 and the genotypes identified as resistant/moderately resistant were further tested both under glass house and field conditions in the subsequent years (2013 and 2014) and seasons (Both *Kharif* and *Rabi*). Based on three years of testing, Phougak, a north eastern landrace with a unique characteristic of clustered panicle was found withconsistent tolerance reaction against

Table1: Disease reaction among rice germplasm under artificial inoculation during 2012 to 2020

S.No	*K-2012(F)*	*R-2013(GH)*	*K-2013(F)*	*R-2014(GH)*	*K-2014(F)*	*K-2017(F)*	*K-2018(F)*	*K-2019(F)*	*K-2020(F)*
Tetep	3	3	3	3	3	3	5	5	3
Phougak	3	3	3	3	3	3	3	3	3
IR-50	9	9	9	9	9	9	9	9	9
Maritchatpi	5	3	5	7	7	NT	NT	NT	NT
TJP-19	3	3	5	7	5	NT	NT	NT	NT
TJP-28	5	3	5	7	7	NT	NT	NT	NT
Remi	3	5	3	5	7	NT	NT	NT	NT
APMS 6B	3	5	5	5	7	NT	NT	NT	NT
495	3	5	5	7	5	NT	NT	NT	NT
APMS-6B	3	5	5	5	7	NT	NT	NT	NT
ARC 6605	5	5	5	7	5	NT	NT	NT	NT
BG-380-2	5	5	5	7	3	NT	NT	NT	NT

NT-Not tested; F-Field; GH-Glass House

sheath blight disease (Dey *et al*., 2016; Dey *et al*., 2019; Dey *et al*., 2020) and its disease reaction under repeated artificial screening in field condition for the past 6 monsoon seasons *viz*., *Kharif*-2012, *Rabi*-2013, *Kharif*-2013, *Rabi*-2014 and *Kharif*-2014 is presented in (Table 1).

Considering its promising performance against sheath blight over the seasons and years, Phougak was evaluated in donor screening nurseries (DSN) of AICRIP during 2019. Sheath blight screening under artificial inoculation conditions was taken up at 16 locations and Phougak showed a disease susceptibility index (*dsi*) of 1.0 at four locations viz., Bankura, Moncampu, Pantnagar and Titabar, a *dsi* of 3.0 at Raipur and 5.0 at remaining locations with an overall mean *dsi* of 4.3 for 9 locations where LSI >4.0 (Table 2.

In addition to its resistance reaction to sheath blight, Phougak was also found promising against leaf blast and neck blast in DSN of AICRIP 2019. Of the 14 locations under artificial screening, Phougak recorded a *dsi* of 1.0 at Rewa, 2.0 at 3 locations *viz*., Gangavati, Mandya, and Ranchi, a *dsi* of 3.0 at 4 locations *viz*., IIRR, Malan, Rajendranagar and Bankura and a *dsi* of 4.0-5.0 at remaining 6 locations. A mean *dsi* of 3.78 was shown by Phougak for 9 locations with LSI>4.0 under artificial inoculation conditions. Natural field screening against leaf blast at 13 locations indicated *dsi* of 1.0 at Rewa, 2.0 at Jagitial, Gangavati and RNC, 3.0 at Almora, Umiam, IMP and Pattambi, 4.0 at Upper Shillong, Ponnampet and Jagdalpur and 5.0 at Mugad and Hazaribhag with a mean *dsi* of 3.71 for 7 locations where LSI>4.0. Multi-location screening both under natural and artificial screening conditions indicated resistance to leaf blast. Screening of Phougak in UBN at ICAR-IIRR confirmed resistance to blast.

Neck blast screening under natural conditions was taken up at 10 locations and Phougak scored a mean *dsi* of 3.3 for 4 locations with a LSI >5.4 and at individual locations, it recorded a *dsi* of 1.0 at Mandya, 2.0 at Umiam, 3.0 at Imphal and 5.0 at remaining 4 locations.

Field and glasshouse screening in multiple years/seasons and multi-location testing under AICRIP-DSN indicated tolerance to sheath blight and resistance to both leaf blast and neck blast in Phougak. In view of its consistent promising performance against sheath blight, leaf blast and neck blast, Phougak was deployed in crosses with elite susceptible cultivars like Swarna, Improved Samba Mahsuri

Table 2: Sheath blight disease reaction in donor screening nursery (DSN) of AICRIP during 2019 under artificial screening conditions

Entry	MND	GNV	LDN	IIRR	CTK	MSD	PTB	ADT	NDL	CHP	RPR	BNK	MNC	PTN	PNT	SI	TTB*
LSI	7.7	7.5	7.0	6.9	6.8	6.8	6.8	6.4	5.7	5.5	5.3	4.8	4.6	4.5	4.4		3.6
Phoghak	5	7	7	5	5	5	5	5	3	5	3	1	1	7	1	4.3	1
T(N)1	9	9	7	9	7	7	9	9	7	7	7	7	7	7	5	7.5	-
IR-50	9	5	7	9	5	7	7	7	7	5	9	7	5	4	5	6.5	-
Tetep	1	3	5	5	3	5	5	3	5	5	3	3	0	5	5	3.7	-

*Titabar with LSI <4.0 was not included in the calculation of DSI for sheath blight.

Table 3: Reaction against Leaf blast in donor screening nursery (DSN) of AICRIP during 2019 under artificial screening conditions

Entry	*MND*	*LNV*	*NLR*	*KJT*	*GNV*	*NWG*	*IIRR*	*MLN*	*RNR*	*SI*
LSI	6.4	6.0	5.7	5.4	5.4	5.1	5.0	4.8	4.4	
Phougak	2	5	6	4	2	6	3	3	3	3.78
Tetep	5	2	4	4	3	3	1	1	3	2.89
HR-12	9	9	6	7	9	8	9	7	9	8.11
TN-1	9	9	7	4	9	7	9	8	9	7.89
IR-64	8	9	5	4	7	5	3	8	7	6.22

Table 4: Reaction against Leaf blast in donor screening nursery (DSN) of AICRIP during 2019 under natural field conditions

Entry	ALM	JGT	HZB	GGT	UMM	USG	PNP	SI
LSI	6.4	5.5	5.4	5.3	4.9	4.6	4.0	
Phougak	3	2	5	5	3	4	4	3.71
Tetep	3	8	1	5	2	5	3	3.85
HR-12	9	6	8	5	-	9	3	6.67
TN-1	9	5	7	5	-	4	3	5.5
IR-64	7	2	3	5	8	3	2	4.29

Table 5: Reaction against neck blast in donor screening nursery (DSN) of AICRIP during 2019 under natural field conditions

Entry	JGT	LNV	UMM	MND	SI
LSI	6.0	5.7	5.7	5.4	
Phougak	5	5	2	1	3.3
HR-12	7	9	-	9	8.3
IR-64	3	9	9	5	6.5
TN-1	5	9	-	7	7.0
Tetep	7	1	3	1	3.0

and Samba Mahsuri and populations are being developed (Tables 3-5).

References

Bhaktavatsalam G, Satyanarayana K, Reddy APK, John VT (1978). Evaluation of sheathblight resistance in rice. *Int. Rice Res. Newsl* **3:** 9–10.

IRRI (2004). Standard evaluation system for rice. International Rice Research Institute, Los Banos, Manila.

Laha GS, Madhav MS, Prakasam V, Jyothi Badri, Ladha Lakshmi (2018-2019). Incentivizing Research in Agriculture: Research Highlights, PP34-35.

Prakasam V, Ladhalakshmi D, Laha GS, Krishnaveni D, Sheshu Madhav M, Jyothi Badri, Srinivas Prasad M and Viraktamath BC (2013). Sheath blight of rice and its management. Technical Bulletin No.72. Directorate of Rice Research (ICAR), Rajendranagar, Hyderabad-500 030, A.P., India. pp. 56.

Prakasam V, Priyanka C, Ravindra Khale, Jyothi Badri, Lydia Ch, Ladha Lakshmi, Laha GS, Prasad MS, Sundaram RM (2020). Comprehensive patho-phenotyping and molecular mapping of RIL population of Wazuhophek/Improved Samba Masuri against sheath blight of rice. 7th International Conference on "Phytopathology in Achieving UN Sustainable Development Goals" during January 16-20 at IARI, New Delhi, India, P 283

Susmita Dey, Badri J, Prakasam V, Bhadana VP, Eswari KB, Laha GS, Priyanka C, Aku R and Ram T. (2016). Identification and agro-morphologicalcharacterization of rice genotypes resistant to sheath blight. *Australas. Plant Pathol.,* **45:** 145-153.

Susmita Dey, Jyothi Badri, Eswari KB and Prakasam V (2020) Diversity analysis for yield traits and sheath blight resistance in rice genotypes. *Electron. J. Plant Breed.,* **11(1):** 66-64.

4. DT-RIL110 (IC0638868; INGR21094), a wheat (*Triticum aestivum* L.) germplasm with drought tolerance.

Sonia Sheoran*, Gyanendra Singh, BS Tyagi, Sindhu Sareen, Rinki, PC Mishra and GP Singh

ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana-132001, India

Email: Sonia.Sheoran@icar.gov.in

The current climatic conditions pose serious threat to wheat production in the form of abiotic stress conditions. Among abiotic stresses, drought stress is of major concern, causing significant yield loss globally. Efforts are underway to mitigate the drought through breeding resilient wheat varieties. The wheat genotype DT-RIL110 was identified as a drought tolerant genotype based on physiological trait characterization.

The genotype DT-RIL110 was developed from the cross Dharwar Dry/DPW621-50 at ICAR-Indian Institute of Wheat & Barley Research (ICAR-IIWBR), Karnal. The parental line DPW621-50 is high yielding genotype released for timely sown irrigated conditions of the NWPZ whereas Dharwar Dry is a well-established drought tolerant Indian genotype (Kirigwi *et al*., 2007). The parental adaptability to endure in the stress condition has been combined in wheatgenotype DT-RIL110. It was phenotyped for two years under drought in rain out shelter (ROS) at Karnal (2016-18) and in rainfed conditions at Powerkheda (2016-17) and Karnal (2018-19) where it showed promising performance for drought stress tolerance as indicated by very low drought susceptibility index (0.48) score (Table 1).

Therefore, this promising genotype (DT-RIL110) was also evaluated under AICRP on Wheat and Barley, 32nd Drought Tolerance Screening Nursery (DTSN) along with 5 checks (DBW 110, C306, K1317, MP3288 and NI5439) at 06 centres (2019-20). The proposed entry DT-RIL110 showed highest tolerance to drought stress with DSI of 0.74 and lowest reduction in yield (18.68%) as compared to five checks in pooled data of 06 drought stress locations (Dharwad, Junagarh, Indore, Kanpur, Bardoli, and Hisar), covering major wheat growing zones (Table 2).This indicated suitability of DT-RIL110 under rainfed environment. DT-RIL110 was also superior to the check varieties for days to heading (DH), days to maturity (DM), plant height(PH), grain filling duration (GFD), grain number per spike (GN/S) and biomass (BM) (Table 2) in pooled data of DTSN conducted at 6 locations.

DH-days to heading, DM-days to maturity, GFD-grain filling duration; PH-plant height; GW/S-grain weight per spike; BM-biomass

It may be concluded that the genotype DT-RIL110 may be used as potential source to be utilizedin wheat breeding programmes to develop drought tolerant bread wheat varieties.

Reference

Kirigwi FM, M Van Ginkel, G Brown-Guedira, BS Gill, GM Paulsen, AK Fritz (2007) Markersassociated with a QTL for grain yield in wheat under drought. *Mol. Breed.* **20:** 401– 413.

5. pau16071 (IC0638869; INGR21095), a wheat (*Triticum aestivum* L.) germplasm pau16071 with leaf rust resistance (LrT) and gluconess (IwT).

Satinder Kaur, Achla Sharma, Puja Srivastva and Parveen Chhuneja*
Punjab Agricultural University, Ludhiana, Punjab-141004, India
**Email: pchhuneja@pau.edu*

The growing and cultivating resistant wheat crop varieties is important in order to meet the demands of the growing population and minimizing the yield losses. The three rust diseases viz stem rust (*Puccinia graminis* f. sp. *tritici*), leaf rust (*Puccinia triticina*), and stripe or yellow rust (*Puccinia striiformis* f. sp. *tritici*) (Aboukhaddour *et al*. 2020; Krattinger *et al.* 2009; Wellings 2011) create a never ending challenges for wheat improvent. Species belonging to the genus *Aegilops* L. are important sources of genetic material for expanding genetic variability of cultivated bread wheat. *Aegilops tauschii*, the D genome donor of wheat, is an invaluable source of genetic variability, which can be utilized for broadening the wheat gene pool. Evaluation of wild *Triticum* and *Aegilops species* at the Punjab Agricultural University, Ludhiana, India has led to the identification of several Aegilops species includingAe. *tauschii* as very good sources of resistance to many diseases including leaf rust and stripe rust (Chhuneja *et al*., 2010).

An accession of *Ae. tauschii* named pau 14195 found to be resistant to mixture of leaf rust races in field and was non-glaucous. Testing at seedling and adut plant stage reveled the rust resistance to be all stage resistance (ASR). Non-Glaucousness found in *Ae. tauschii* is a visual trait, which is related to greyish or whitish appearance on the leaves, sheaths, glumes and stems in wheat. This appearance is due to epicuticular wax exudates produced by the plant. Cuticular wax deposits are known to reduce the loss of water due to transpiration and hence increasing drought tolerance in plants (Riederer and Schreiber 2001). This accession of *Ae. tauschii* was crossed with *T. durum* cultivar PBW114 and the F_1 so obtained was crossed with hexaploid wheat WH542. The double F_1 was again backcrossed twice with WH542 to obtain BC_3F_1 *Triticum durum* cv. PBW114/ *Ae. tauschii* acc. pau14195//4**T. aestivum* cv. WH542. Linked leaf rust resistance and non-glaucousness genes from *Ae. tauschii* to cultivated wheat variety WH542 were mapped. Inheritance studies was done on F_2 population from a BC_3 plant derived from the cross *Triticum durum* cv. PBW114/ *Ae. tauschii* acc. pau14195//4**T. aestivum* cv. WH542 against leaf rust race 77-5 at seedling stage and against mixture of leaf rust races at adult plant stage. The rust data revealed monogenicdominant inheritance for both the traits of rust resistance and non-glaucousness. The leaf rust resistance and the non-glaucousness gene were tentatively named *LrT* and *IwT*, respectively. SSR markers *Xbarc124, Xgdm5, Xgdm35, Xcfd51* and EST-derived markers *Xcau96* and

Table 1: Drought susceptibility index (DSI) under drought stress (pooled over four environments)

Genotype	*Karnal * (2016-17)*	*Powarkheda (2016-17)*	*Karnal * 2017-18*	*Karnal 2018-19*	*Pooled Data*
DT-RIL110	0.47	-0.01	0.65	0.79	0.48
Dharwar Dry ©	-0.06	0.9	0.78	0.91	0.63
AKAW3717 ©	1.02	0.91	0.98	0.83	0.94
C306 ©	0.95	0.89	0.87	0.99	0.93
DPW621-50	1.63	1.3	1.36	1.02	1.33

*ROS-Rain out shelter

Table 2: Pooled analysis of DSI and agro-morphological traits of DTSN genotypes during 2019-20

Trait	*Test Genotype*	*Checks*				
	DT-RIL110	DBW110 ©	C306 ©	K1317©	MP3288©	NI5439©
Pooled DSI	0.745	0.81	0.833	0.954	1.36	1.44
Yield % reduction	18.68	20.36	20.88	23.9	33.97	36.19
DH	64	74	71	67	68	71
DM	111	114	115	115	112	115
GFD	47	40	44	44	44	44
PH	72	81.3	93.4	89	84.6	85.3
GW/S	1.74	1.75	1.58	2.3	1.95	1.65
BM (g)	1862	1769	1734	1771	1507	1679

Xte6 on chromosome 2DS were linked with both genes. Chromosomal assignments of the genes were confirmed by testing linked SSR markers on Chinese Spring nullitetrasomics lines. SSR markers *Xcau96* (1.6 cM) and *Xbarc124* (0.6cM) flanked *LrT* and *Xgdm35* (4.1 cM) and *Xte6* (2.5 cM) flanked non-glaucousness gene. *LrT* and *IwT* showed a recombination distance of 3.4 cM. Hence, *IwT* can be used as an easy to score morphological marker of *LrT* during its transfer to other glaucous backgrounds.

References

Aboukhaddour R, Fetch T, McCallum B D, Harding M W, Beres B L and Graf R J (2020) Wheat diseases onthe prairies: A Canadian story. *Plant Pathol. J.* **(October 2019):** 1–15.

Chhuneja P, Garg T, Kumar R, Kaur S, Sharma A, Bains NS, Ahuja M, Dhaliwal HS and Singh K (2010) Evaluation of Aegilops tauschii Coss. germplasm for agromorphological traits and genetic diversity using SSR loci. *Indian J. Genet.* **70:** 328–338.

Krattinger S G, Lagudah E S, Spielmeyer W, Singh R P, Huerta-espino J, Mcfadden H, Bossolini E, Selter L L and Keller B (2009) Pathogens in *Wheat sci. (80-)* **323(February):** 1360–1363.

Riederer M and Schreiber L (2001) Protecting against water loss: analysis of the barrier properties of plant cuticles. Journal of Experimental Botany **52:** 2023–2032.

Wellings C R (2011) Global status of stripe rust: A review of historical and current threats. **179(1):** 129–141.

6. pau 16068 (IC0638873; INGR21096), a wheat (*Triticum aestivum* L.) germplasm with resistance to powdry mildew and two powdry mildew resistance genes PmTb7A.1 and PmTbA.2 mapped on chromosome 7AL.

Parveen Chhuneja*, Kuldeep Singh and Satinder kaur

Punjab Agricultural University, Ludhiana, Punjab-141004, India

**Email: pchhuneja@pau.edu*

Powdery mildew (PM), caused by *Blumeria graminis* f. sp. *tritici*, is one of the important wheat diseases, worldwide. A number of PM resistance genes have been identified from cultivated wheat and its wild relatives. So far more than 82 PM resistance genes/alleles have been identifiedat 50 loci (*Pm1-Pm53*, *Pm18- Pm1c*, *Pm22 - Pm1e*, *Pm23 - Pm4c*, *Pm31 - Pm21*) in wheat and its wild relatives (McIntosh *et al* 2013). Of the 50 loci, 11 have been mapped on the A genome, 26 on the B genome and 13 on the D genome of wheat. Twenty-seven these genes/alleles have been transferred into wheat from wild species (Peterson *et al* 2015, Xiao *et al* 2013). In India, PM is prevalent in the northern and southern hill zones causing serious yield losses whereas in north western plains zone (NWPZ) of India, which constitutes the most productive wheat growing region, PM appears sporadically but causing significant yield losses (Singh *et al* 2009).

T. boeoticum, (2n = 2x = 14, AA), a close relative of the A genome donor of wheat, harbours useful variability for many agronomically important traits including resistance to diseases (Feldman and Sears 1981, Singh *et al* 2007). We identified *T. boeoticum* acc. pau5088 having two PM resistance genes, designated as *PmTb7A.1* and *PmTb7A.2*, and mapped on chromosome 7AL approximately 48cM apart. Two resistance gene analogue (RGA)-STS markers Ta7AL- 4556232 and 7AL-4426363 were identified to be linked to the *PmTb7A.1* and *PmTb7A.2*, at a distance of 0.6cM and 6.0cM, respectively. Following marker assisted selection (MAS), the two genes were transferred to *T. aestivum* using *T. durum* as bridging species. As many as 12,317 florets of F_1 of the cross *T. durum /T. boeoticum* were pollinated with *T. aestivum* lines PBW343-IL and PBW621 to produce 61 and 65 seeds, respectively, of three-way F_1. Theresulting F_1s of the cross *T. durum/T. boeoticum// T. aestivum* were screened with markerflanking both the PM resistance genes *PmTb7A.1* and *PmTb7A.2* (foreground selection) and the selected plants were backcrossed to generate BC_1F_1. Marker assisted selection was carried bothin BC_1F_1 and the BC_2F_1 generations. Introgression of alien chromatin in BC_2F_1 plants varied from 15.4 - 62.9 percent. Out of more than 110 BC_2F_1 plants showing introgression for markers linked to the two PM resistance genes, 40 agronomically desirable plants were selected for background selection for the carrier chromosome to identify the plants with minimum of the alien introgression. Cytological analysis showed that most plants have chromosome number ranging from 40-42. The BC_2F_2 plants homozygous for the two genes have been identified. These will be crossed to generate lines combining both the PM resistance genes but withminimal of the alien introgression. The PM resistance gene *PmTb7A.1* maps in a region very close to *Sr22*, a stem rust resistance gene effective against the race Ug99. Analysis of selected plants with markers linked to *Sr22* showed introgression of *Sr22* from *T. boeoticum* in several BC_2F_1 plants. Thus, in addition to PM resistance, these progenies might also carry resistance to stem rust race Ug99.

References

McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Appels R, Xia XC (2013) Catalogue of gene symbols forwheat: 2014 supplement. Available: *http://www.shigen.nig.ac.jp/wheat/komugi/genes/macgene/ supplement2013.pdf.*

Petersen S, Lyerly JH, Worthington ML, Parks WR, Cowger C, Marshall DS, *et al*. (2015). Mapping of powdery mildew resistance gene Pm53 introgressed from Aegilops speltoides into soft red winter wheat. *Theor. Appl. Genet.* **128:** 303–312. doi: 10.1007/s00122-014- 2430-8 PMID: 25425170

Xiao M, Song F, Jiao J, Wang X, Xu H, Li H (2013) Identification of

the gene Pm47 on chromosome 7BS conferring resistance to powdery mildew in the Chinese wheat landrace Hongyanglazi. *Theor. Appl. Genet*. **126:** 1397–1403. *doi: 10.1007/s00122-013-2060-6 PMID: 23429903*

Singh DP, Sharma AK, Singh D, Rana SK, Singh KP, Srivastava K, *et al*. (2009) Resistance to powdery mildew in Indian wheat. *Pl Dis Res*: **24:** 942.

Feldman M, Sears ER (1981) The wild gene resources of wheat. *Sci Am* **244:** 98–109.Feld

Singh K, Ghai M, Garg M, Chhuneja P, Kaur P, Schnurbusch T, *et al* (2007b) An integrated molecular linkage map of diploid wheat based on a *Triticum boeoticum* X T. monococcumRIL population. *Theor. Appl. Genet.* **115:** 301–312. PMID: 17565482

7. IC252458 (IC252458; INGR21097), a wheat (*Triticum aestivum* L.) germplasm with three minor/adult plant rust resistance genes (APR) for leaf rust.

Vikas VK*[1], Sundeep Kumar[2], BS Phogat[2], AK Sharma[3], MS Saharan[4], Amit Kumar Singh[2], Jyoti Kumari[2], Rakesh Singh[2], Sherry R Jacob[2], M Sivasamy[1], P Jayaprakash[1], JP Jaiswal[5], Deepsikha[5], SP Singh[6], IK Kalappanavar[7], SS Vaish[8], PC Mishra[9] and GP Singh[3]

[1]*ICAR-IARI Regional Station, Wellington, Tamil Nadu-643231, India*

[2]*ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012, India*

[3]*ICAR-Indian Institute of Wheat & Barley Research, Karnal, Haryana-132001, India*

[4]*ICAR-Indian Agricultural Research Institute, Pusa Campus, New Delhi-110012, India*

[5]*Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Uttarakhand-263145, India*

[6]*Narendra Deva University of Agriculture and Technology, Faizabad, Uttar Pradesh-224229, India*

[7]*University of Agricultural Sciences, Dharwad, Karnataka-580005, India*

[8]*Banaras Hindu University, Varanasi-221005, Uttar Pradesh-560065, India*

[9]*Zonal Agricultural Research Station (JNKVV), Powarkheda, Madhya Pradesh-461110, India*

**Email: vkvikaswtn@gmail.com*

Leaf rust (*Puccinia triticina* Eriks.) is a fungal disease of wheat (*Triticum aestivum* L.), which causes considerable yield loss. Adult plant resistance (APR) is one of the most sustainableapproaches to control leaf rust. A comprehensive germplasm evaluation study of wheat accessions conserved in the Indian National Genebank was conducted to identify sources of minor/adult plant rust resistance genes (APR) for leaf rust at phenotypic and genotypic level. Field phenotyping was carried out across ten different locations viz., 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 thepresence of APR genes, *Lr34*+, *Lr46*+, *Lr67*+ and *Lr68* in Indian wheat germplasm. In field screening, 190 wheat accessions were selected from 6,319 accessions based on leaf tip necrosis (LTN), disease severity and the average coefficient of infection. APR genes are also associated with leaf tip necrosis (LTN), a phenotypical marker that is linked or pleiotropic with these genes [14]. LTN appears on the flag leaves when plants are grown in fields (Lagudah *et al*., 2006). Molecular screening of 190 wheat accessions revealed that 73% of the accessions possessed known APR genes either as single or as a combination of two or three genes. Furthermore, 49 accessions possessing two and three APR genes were evaluated for yield stability across four different viz., Pantnagar, Varanasi, Powarkheda and Pune, using additive main effects andmultiplicative interaction (AMMI) model (Kumar *et al*., 2019).

Among the nine wheat accessions identified with the presence of three APR genes, wheat accession, IC252458 showed the presence of three adult plant leaf rust resistance genes, *Lr34*+ (*Lr34/Sr57/Yr18/Pm38/Ltn1*), *Lr46*+ (*Lr46/Sr58/Yr29/Pm39/Ltn2*) and *Lr67*+ (*Lr67/Yr46/Sr55/Pm46/Ltn3*). Accession, IC252458 displayed resistance reaction (0) in all the locations with average coefficient of Infection (ACI) of 0.0. Moreover, APR genes have pleiotropic association with stem and stripe rust resistance along with powdery mildew which is an added advantage. Synergistic combination of minor rust resistance genes made the accession resistance to the prevailing leaf rust pathotypes in India (Singh *et al*., 1992). APR genes are pathotype non-specific resistance based on horizontal type of resistance mechanism. Development of new pathotype(s) is slow/remote compared to major gene resistance which leadsto durable resistance. So, the germplasm could be vital source for imparting durable rust resistance in wheat.

References

Lagudah ES, H Mc Fadden, RP Singh, J Huerta-Espino, HS Bariana and W Spielmeyer (2006)Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. *Theor. Appl. Genet.* **114:** 21-30.

Kumar S, BS Phogat, VK Vikas, AK Sharma, MS Saharan, AK Singh *et al*. (2019) Mining ofIndian wheat germplasm collection for adult plant resistance to leaf rust. *PLoS One* **14(3):** e0213468

Singh RP, S Rajaram (1992) Genetics of adult plant resistance to leaf rust in 'Frontana' andthree CIMMYT wheats. *Genome* **35:** 24-31.

8. IC290150 (IC290150; INGR21098), a wheat (*Triticum aestivum* L.) germplasm with three minor/adult plant rust resistance genes (APR) for leaf rust.

Vikas VK*[1], Sundeep Kumar[2], BS Phogat[2], AK Sharma[3], MS Saharan[4], Amit Kumar Singh[2], Jyoti Kumari[2], Rakesh Singh[2], Sherry R Jacob[2], M Sivasamy[1], P Jayaprakash[1], JP Jaiswal[5], Deepsikha[5], SP Singh[6], IK Kalappanavar[7], SS Vaish[8], PC Mishra[9] and GP Singh[3]

[1]*ICAR-IARI Regional Station, Wellington, Tamil Nadu-643231, India*

[2]*ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012, India*

[3]*ICAR-Indian Institute of Wheat & Barley Research, Karnal, Haryana-132001, India*

[4]*ICAR-Indian Agricultural Research Institute, Pusa Campus, New Delhi-110012, India*

[5]*Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Uttarakhand-263145, India*

[6]*Narendra Deva University of Agriculture and Technology, Faizabad, Uttar Pradesh-224229, India*

[7]*University of Agricultural Sciences, Dharwad, Karnataka-580005, India*

[8]*Banaras Hindu University, Varanasi-221005, Uttar Pradesh-560065, India*

[9]*Zonal Agricultural Research Station (JNKVV), Powarkheda, Madhya Pradesh-461110, India*

**Email: vkvikaswtn@gmail.com*

Leaf rust (*Puccinia triticina* Eriks.) is a fungal disease of wheat (*Triticum aestivum* L.), which causes considerable yield loss. Adult plant resistance (APR) is one of the most sustainable approaches to manage leaf rust. APR is best expressed at the adult plant stage wherein resistance is conferred by multiple additive genes possessing quantitative inheritance. Since APR is generally governed by multiple additive genes, it is not subjected to the "boom and bust cycle" of disease epidemics. Therefore, APR is considered more durable than all stage resistance (ASR) or seedling resistance or race-specific resistance, which is governed by a major gene providing hypersensitive response (HR).

A comprehensive germplasm evaluation study of wheat accessions conserved in the Indian National Genebank was conducted to identify sources of minor/adult plant rust resistance genes (APR) for leaf rustat phenotypic and genotypic level. Field phenotyping was carried out across ten different locations *viz*., 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. In field screening, 190 wheat accessions were selected from 6,319 accessions based on leaf tip necrosis (LTN), disease severity and the average coefficient of infection. APR genes are also associated with leaf tip necrosis (LTN), a phenotypical marker that is linked or pleiotropic with these genes. LTN appears on the flag leaves when plants are grown in fields (Lagudah *et al*., 2006). Molecular screening of 190 wheat accessions revealed that 73% of the accessions possessed known APR genes either as single or as acombination of two or three genes (Kumar *et al*., 2019). Among the nine wheat accessions identified with the presence of three APR genes, wheat accession, IC290150 showed the presence of three adult plant leaf rust resistance genes, *Lr34+* (*Lr34/Sr57/Yr18/Pm38/Ltn1*), *Lr67+* (*Lr67/Yr46/Sr55/Pm46/Ltn3*) and *Lr68*. Accession, IC290150displayed resistance reaction (5R) in all the locations with average coefficient of Infection (ACI) of 0.7. Moreover, APR genes have pleiotropic association with stem and stripe rust resistance along withpowdery mildew which is an added advantage. Synergistic combination of minor rust resistance genes made the accession resistance to the prevailing leaf rust pathotypes in India (Singh *et al*., 1992). APR genes are pathotype non-specific resistance based on horizontal type of resistance mechanism. Development of new pathotype(s) is slow/remote compared to major gene resistance which leads to durable resistance. Resistance based on APR genes, in the background of high yielding cultivars, is expected to provide a high level of race non-specific resistance, which is durable. So, the germplasm could be vital source for imparting durable rust resistance in wheat.

References

Kumar S, BS Phogat, VK Vikas, AK Sharma, MS Saharan, AK Singh *et al*. (2019) Mining of Indian wheatgermplasm collection for adult plant resistance to leaf rust. *PLoS One* **14(3):** e0213468

Lagudah ES, H Mc Fadden, RP Singh, J Huerta-Espino, HS Bariana and W Spielmeyer (2006)Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. *Theor. Appl. Genet.* **114:** 21-30.

Singh RP, S Rajaram (1992) Genetics of adult plant resistance to leaf rust in 'Frontana' and three CIMMYT wheats. *Genome* **35:** 24-31.

9. IC279875 (IC279875; INGR21099), a wheat (*Triticum aestivum* L.) germplasm with two minor/adult plant rust resistance genes (APR) for leaf rust.

Vikas VK*[1], Sundeep Kumar[2], BS Phogat[2], AK Sharma[3], MS Saharan[4], Amit Kumar Singh[2], Jyoti Kumari[2], Rakesh Singh[2], Sherry R Jacob[2], M Sivasamy[1], P Jayaprakash[1], JP Jaiswal[5], Deepsikha[5], SP Singh[6], IK Kalappanavar[7], SS Vaish[8], PC Mishra[9] and GP Singh[3]

[1]*ICAR-IARI Regional Station, Wellington, Tamil Nadu-643231, India*

[2]*ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012, India*

[3]*ICAR-Indian Institute of Wheat & Barley Research, Karnal, Haryana-132001, India*

[4]*ICAR-Indian Agricultural Research Institute, Pusa Campus, New Delhi-110012, India*

[5]*Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Uttarakhand-263145, India*

[6]*Narendra Deva University of Agriculture and Technology, Faizabad, Uttar Pradesh-224229, India*

[7]*University of Agricultural Sciences, Dharwad, Karnataka-580005, India*

[8]*Banaras Hindu University, Varanasi-221005, Uttar Pradesh-560065, India*

[9]*Zonal Agricultural Research Station (JNKVV), Powarkheda, Madhya Pradesh-461110, India*

**Email: vkvikaswtn@gmail.com*

Leaf rust (*Puccinia triticina* Eriks.) is a fungal disease of wheat (*Triticum aestivum* L.), which causes considerable yield loss. Adult plant resistance (APR) is one of the most sustainable approaches to control leaf rust. APR is best expressed at the adult plant stage wherein resistance is conferred by multiple additive genes possessing quantitative inheritance. Since APR is generally governed by multiple additive genes, it is not subjected to the "boom and bust cycle" of disease epidemics. Therefore, APR is considered more durable than all stage resistance (ASR) or seedling resistance or race-specific resistance, which is governed by a major gene providing hypersensitive response (HR).

A comprehensive germplasm evaluation study of wheat accessions conserved in the Indian National Genebank was conducted to identify sources of minor/adult plant rust resistance genes (APR) for leaf rustat phenotypic and genotypic level. Field phenotyping was carried out across ten different locations *viz.,* 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. In field screening, 190 wheat accessions were selected from 6,319 accessions based on leaf tip necrosis (LTN), disease severity and the average coefficient of infection. APR genes are also associated with leaf tip necrosis (LTN), a phenotypical marker that is linked or pleiotropic with these genes. LTN appears on the flag leaves when plants are grown in fields (Lagudah *et al*., 2006). Molecular screening of 190 wheat accessions revealed that 73% of the accessions possessed known APR genes either as single or as acombination of two or three genes. (Kumar *et al*., 2019).

Among the nine wheat accessions identified with the presence of three APR genes, wheat accession, IC279875 showed the presence of two adult plant leaf rust resistance genes, *Lr34+* (*Lr34/Sr57/Yr18/Pm38/Ltn1*) and *Lr68***.** Accession, IC279875 displayed resistance reaction (0) in all the locations with average coefficient of Infection (ACI) of 0.0. Moreover, APR genes have pleiotropic association with stem and stripe rust resistance along with powdery mildew which is an added advantage. Synergistic combination of minor rust resistance genes made the accession resistance to the prevailing leaf rust pathotypes in India (Singh *et al*., 1992). APR genes are pathotype non-specific resistance basedon horizontal type of resistance mechanism. Development of new pathotype(s) is slow/remote comparedto major gene resistance which leads to durable resistance. Resistance based on APR genes, in the background of high yielding cultivars, is expected to provide a high level of race non-specific resistance, which is durable. So, the germplasm could be vital source for imparting durable rust resistance in wheat.

References

Kumar S, BS Phogat, VK Vikas, AK Sharma, MS Saharan, AK Singh *et al*. (2019) Mining of Indian wheatgermplasm collection for adult plant resistance to leaf rust. *PLoS One* **14(3):** e0213468

Lagudah ES, H Mc Fadden, RP Singh, J Huerta-Espino, HS Bariana and W Spielmeyer (2006)Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. *Theor. Appl. Genet.* **114:** 21-30.

Singh RP, S Rajaram (1992) Genetics of adult plant resistance to leaf rust in 'Frontana' and three CIMMYT wheats. *Genome* **35:** 24-31.

10. DWRB 206 (DWRNB 17) (IC0638874; INGR21100), a barley (*Hordeum vulgare* L.) germplasm with resistant to stripe rust at APR under artificial inoculation in naked barley.

Jogendra Singh*, Sudheer Kumar, Lokendra Kumar, Dinesh Kumar, Chuni Lal, Rekha Malik, AS Kharub, RPS Verma and GP Singh

ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana-132001, India

**Email: Jogendra.singh2@icar.gov.in*

DWRB206 (DWRNB17) is a six-row hulless barley genotype, selected from exotic (INBYT-(2013)-20, ICARDA) breeding material (ZIGZIG/4/TOCTE//HIGO/LINO/3/PETUNIA1). DWRB206 was evaluated for agro-morphological traits at ICAR-IIWBR, Hisar and for disease reaction (stripe rust at 5 locations) during 2016-17 in Initial Barley Disease Screening Nursery (IBDSN) under artificial epiphytotic conditions. The genotype shown higher resistance against stripe rust (ACI=3.0 HS 10S) at adult plant stage (Table 1). The check cultivars as well as infector showed high susceptible reaction against stripe rust. The genotype was again screened for stripe rust at 6 locations in National Barley Disease Screening Nursery (NBDSN) during 2018-19. DWRB206 exhibited resistance to stripe rust with ACI=5.6 and HS=10S only, while the huskless checks Karan16 and NDB943 were highly susceptible.

Table 1: Disease reaction of DWRB206 (DWRNB17) in IBDSN (2016-17) and NBDSN (2018-19)

Genotype	*Stripe rust reaction*			
	IBDSN (2016-17)		*NBDSN (2018-19)*	
	ACI	*HS*	*ACI*	*HS*
DWRB206 (DWRNB17)	3.0	10S	5.6	10S
Karan16	NT	NT	46.6	60S
NDB 943	NT	NT	66.6	100S
Infector	84	100S	83.3	100S

ACI= average coefficient of infection, HS= highest score across all locations

Agro-morphological and quality traits

DWRB206 flowers in 95 days and matures in 127 days. Its average plant height is 111 cm and has 109 tillers/meter. The average spike length is 8.0 cm with 68 grains per spike and thousand grain weight of 36.5g. The genotype had 61.3 kg/hl test weight and protein content 12.9%.

References

Anonymous (2017). Progress report of AICRP on Wheat and Barley 2016-17, Barley Improvement. Eds. AS Kharub, Chuni Lal, Dinesh Kumar, Jogendra Singh, Lokendra Kumar, Anil Khippal, Vishnu Kumar, Sudheer Kumar, SC Bhardwaj, Poonam Jasrotia, Rekha Malik, Ajay Verma, Satyavir Singh and GP Singh. ICAR-Indian Institute of Wheat and Barley Research, Karnal, India pp. 3.2-3.14.

Anonymous (2019). Progress report of AICRP on Wheat and Barley 2018-19, Barley Improvement. Eds. AS Kharub, Chuni Lal, Dinesh Kumar, Jogendra Singh, Vishnu Kumar, Amit Kumar Sharma, Anil Khippal, Sudheer Kumar, SC Bhardwaj, Poonam Jasrotia, Rekha Malik, Ajay Verma, Satyavir Singh and GP Singh. ICAR-Indian Institute of Wheat and Barley Research, Karnal, India pp. 3.13-3.14.

11. BCLA11-6 (IC0638875; INGR21101), a barley (*Hordeum vulgare* L.) germplasm with corn leaf aphid resistance genotype.

Rekha Malik*, Poonam Jasrotia, RPS Verma, AS Kharub, Dinesh Kumar, Jogendra Singh, Lokendra Kumar, Chuni Lal, Surendra Singh, Rajendra Kumar and GP Singh

ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana-132001, India

**Email: rekha.malik@icar.gov.in*

BCLA11-6 is a six-row barley genotype, developed from a cross between BCU390/Alfa93 in institutional barley breeding program at IIWBR, Karnal in barley network program and fixed up to F9 generation. This genotype was found resistant for corn leaf aphid under epiphytotic and natural infection conditions. BCLA11-6 was screened for five consecutive years (2014-19) in especially designed structure in net house under high load of Corn leaf aphid (CLA) inoculum (Table 1). The genotype was consistently found resistant for CLA during 2014-19. It was then contributed to the multilocation testing under AICRP Wheat and Barley in NBDSN in 2019-20 under natural hot spot condition (Table 2). BCLA11-6 has shown average resistance (1.7) in 2019-20 at two locations and almost similar resistance score (1.67) during 2014-19 at IIWBR, Karnal on a 1 to 5 scale where 1 is immune and 5 is highly susceptible. The genotype was evaluated for agro-morphological traits at ICAR-IIWBR, Karnal during 2018-20.

Agro-morphological and quality traits: BCLA11-6 flowers in 95 days and matures in 145 days. Its average plant height is 159 cm. The flag leaf waxiness is present (low) and spike

Table 1: Screening of BCLA11-6 against foliar aphids under epiphytotic conditions during 2014-19 at ICAR-IIWBR, Karnal

	Location: karnal											
	2014-15		*2015-16*		*2016-17*		*2017-18*		*2018-2019*		*Average of fiveyears*	
Entry no.	*Average number of aphids/ tiller during the season*	*Grade*	*Average number of aphids/ tiller during the season*	*Grade*	*Average number of aphids/ tiller during the season*	*Grade*	*Average number of aphids/ tiller during the season*	*Grade*	*Average number of aphids/ tiller during the season*	*Grade*	*Overall average number of aphids/ tiller*	*Grade*
BCLA11-6	1.61	*R	1.63	R	1.81	R	1.69	R	1.63	R	1.67	R
Alfa –93 (Susceptible Check)	117.57	**HS	119.03	HS	129.63	HS	114.13	HS	111.32	HS	118.33	HS

*Resistant; **Highly Susceptible

Table 2: Screening of BCLA11-6 against foliar aphids under natural hot spot conditions during 2019-20 in coordination trial at Karnal and Kanpur

Entry No.	Kanpur					Karnal					Overall Av. score	Overall HS
	Date of observation			Av. score	HS	Date of observation			Av. score	Highest score		
	05.02.20	12.02.20	19.02.20			03.02.20	17.02.20	02.03.20				
BCLA 11-6	1	2	1	1.3	2	2	2	2	2.0	2	1.7	2
Alfa - 93	5	5	5	5.0	5	5	5	5	5.0	2	5.0	5

waxiness is absent. Leaf width is broad, spike length is medium. Auricle pigmentation is absent. The thousand grain weight is 42.5g.

References

Anonymous (2020). Progress Report of AICRP on Wheat & Barley 2019-20, Barley Improvement. Eds: RPS Verma, AS Kharub, Chuni Lal, Dinesh Kumar, Jogendra Singh, Amit Kumar Sharma, Anil Khippal, Sudheer Kumar, SC Bhardwaj, Poonam Jasrotia, Rekha Malik, Ajay Verma, Satyavir Singh and GP Singh. ICAR-Indian Institute of Wheat andBarley Research, Karnal, India. P. 215

Rekha Malik, Poonam Jasrotia, Surendra Singh1, Rajendra Kumar, Ajit Singh Kharub and RPS Verma (2019). Identification of promising barley genotypes with aphid resistance. Wheat and Barley Newsletter, ICAR-IIWBR, Karnal. July-Dec, 2019: 19-20.

12. BCLA3 (IC0638876; INGR21102), a barley (*Hordeum vulgare* L.) germplasm with corn leaf aphid resistance genotype.

Rekha Malik*, Poonam Jasrotia, RPS Verma, AS Kharub, Dinesh Kumar, Jogendra Singh, Lokendra Kumar, Chuni Lal, Rajendra Kumar, Surendra Singh and GP Singh

ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana-132001, India

**Email: rekha.malik@icar.gov.in*

BCLA3 is a two-row barley genotype, developed from a cross between EB921/Alfa93 in institutional barley breeding program at IIWBR, Karnal in barley network program and fixed up to F9 generation. Thisgenotype was found resistant for corn leaf aphid under epiphytotic and natural infection conditions. BCLA3 was screened for five consecutive years (2014-19) in especially designed structure in net house under high load of Corn leaf aphid (CLA) inoculums. The genotype was consistently found resistant for CLA during 2014-19. It was then contributed to the multilocation testing under AICRP Wheat and Barley in NBDSN in 2019-20 under natural hot spot conditions. BCLA3 has shown average resistance (1.7) in 2019-20 at two locations and almost similar resistance score (1.60) during 2014-19 at IIWBR, Karnal on a1 to 5 scale where 1 is immune and 5 is highly susceptible. The genotype was evaluated for agro-morphological traits at ICAR-IIWBR, Karnal during 2018-20.

Table 1: Screening of BCLA3 against foliar aphids under epiphytotic conditions during 2014-19 at ICAR-IIWBR, Karnal

Entryno.	Location: karnal											
	2014-15		2015-16		2016-17		2017-18		2018-2019		Average of five years	
	Average numberof aphids/tiller during the season	Grade	Average number of aphids/ tiller during the season	Grade	Average number of aphids/ tiller during the season	Grade	Average number of aphids/ tiller during the season	Grade	Average number of aphids/ tiller during the season	Grade	Overall average number of aphids/ tiller	Grade
BCLA3	1.47	*R	1.68	R	1.75	R	1.56	R	1.54	R	1.60	R
Alfa –93 (Susceptible Check)	117.57	**HS	119.03	HS	129.63	HS	114.13	HS	111.32	HS	118.33	HS

*Resistant; **Highly Susceptible

Table 2: Screening of BCLA3 against foliar aphids under natural hot spot conditions during 2019- 20 in coordination trial at Karnal and Kanpur

Entry No.	Kanpur					Karnal						
	Date of observation					Date of observation						
	05.02.20	12.02.20	19.02.20	Av. score	HS	03.02.20	17.02.20	02.03.20	Av. score	Highest score	Overall Av. score	Overall HS
BCLA3	1	2	1	1.3	2	2	2	2	2.0	2	1.7	2
Alfa - 93	5	5	5	5.0	5	5	5	5	5.0	2	5.0	5

Agro-morphological and quality traits

BCLA3 flowers in 99 days and matures in 146 days. Its average plant height is 124 cm. The flag leaf waxiness is present (low) and spike waxiness is absent. Leaf width and spike length is medium. Auricle pigmentation is present. The thousand grain weight is 44.0g.

References

Anonymous (2020). Progress Report of AICRP on Wheat & Barley 2019-20, Barley Improvement. Eds: RPS Verma, AS Kharub, Chuni Lal, Dinesh Kumar, Jogendra Singh, Amit Kumar Sharma, Anil Khippal, Sudheer Kumar, SC Bhardwaj, Poonam Jasrotia, Rekha Malik, Ajay Verma, Satyavir Singh and GP Singh. ICAR-Indian Institute of Wheat and Barley Research, Karnal, India. P. 215

Rekha Malik, Poonam Jasrotia, Surendra Singh1, Rajendra Kumar, Ajit Singh Kharub and RPS Verma (2019). Identification of promising barley genotypes with aphid resistance. *Wheat and Barley Newsletter,* ICAR-IIWBR, Karnal. July-Dec, 2019:19-20.

13. IC340947 (IC0340947; INGR21103), a french bean (*Phaseolus vulgaris* L.) germplasm resistant to BCMV disease.

Basavaraja T[*1], Manjunatha L[1], Mohar Singh[2], Rahul Chandora[2], Shiv Sewak[1] and NP Singh[1]

[1]*ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh-208024, India.*

[2]*ICAR-NBPGR Regional Station, Shimla, Himachal Pradesh-171004, India.*

[*]*Email: Basavaraja.T@icar.gov.in*

Rajmash/Common bean (*Phaseolus vulgaris* L.; 2*n* = 2*x* = 22) is also known by kidney bean, French bean, Snap bean and dry bean etc. It is one of the most vital and highly relished pulse crop used for direct human consumption globally and is an important component of subsistence agriculture (Broughton *et al*., 2003). Therefore, rajmash is regardedas "Grain of hope". In India, rajmash mainly produced by resource-poor farmers, small and marginal land holding farmers in the traditional production system that include rotation with vegetables and intercropping of climbing/ pole type varieties with grain amaranth, potato and maize during *Kharif* season in North Eastern Hilly (NEH) region. While, it grown as a sole crop by using bush types varieties during *rabi* season in northern plain and central India. It is growing under

diverse geographical location in which crop may expose to varied climatic condition. However, crop was prone to many diseases, among them Bean common mosaic virus (BCMV) is one of the most destructive diseases of common bean, it is mainly transmitted by vector Aphid and having seed borne nature. The virus belongs to genus *Potyvirus* of family *Potyviridae* and in India, BCMV is of regular recurrence in North- western Himalayas with disease incidence ranging from 0.5 to 77.0 % (Sharma *et al.* 1998; Kapil *et al.* (2011)). Thus, the prevalence of BCMV disease has posed a serious threat for dry bean production in rajmash growing areas of Himachal Pradesh, Jammu & Kashmir, Sikkim, Uttar Pradesh and Uttarkhand. Hence, Identification of stable BCMV resistant donors is crucial in Rajmash/Common bean improvement programme.

Pedigree

During *rabi* 2015-16, 2016-17, 2017-18 and 2018-19 a set of 300 germplasm accessions were evaluated for agronomic traits and BCMV disease screening under natural epiphytotic condition at IIPR, Kanpur by using BCMV resistant checks as Arun & Amber while, susceptible check as Jawala, Uday and IC341339. Disease scoring was done at three stages of crop growth viz., 45 DAS, 65 DAS and 90 DAS (Pathologist assistance involved). In these experiments, both agronomic and morphological data were recorded and based on BCMV percent disease incidence (PDI) data revealed that germplasm accession *viz.,* IC340947 and IC360831 were highly resistant against BCMV disease as they recorded 0.00% PDI over four consecutive seasons and *Kharif* rajmash is a highly remunerative pulse crop in hilly tract of Himachal Pradesh but BCMV disease is a major obstacle for grain production. During *Kharif* 2017 and 2018, a set of selected promising germplasm accession were screened against BCMV disease under natural field condition at NBPGR, Regional station, Shimla by using BCMV resistant checks as Arun & Amber while, susceptible check as Jawala and Uday. Based on agronomic traits and BCMV disease reaction scale the germplasm accession *viz.,* IC340947 and IC360831 were superior to check varieties and highly resistant against BCMV disease as they recorded 0.00% PDI. In addition to that, during *rabi* 2019-20 a set of selected promising germplasm accessions were screened against BCMV disease both under field and lab condition through sap inoculation method, similarly above experiments both agronomic and BCMV disease reaction was recorded at three different stages of crop growth. Likewise, previous experiments the germplasm accessions such as IC340947 and IC360831 were highly consistent and noticed highly resistant reaction against BCMV disease and stable across the location and seasons. Therefore, these donors being already utilized in disease breeding programme of Common bean. The germplasm identification committee under the chairmanship of Director, ICAR-IIPR visited the field on 09.03.2020 and examined the BCMV resistant donor in all aspects and GIC committee recommended for submission of germplasm registration proposal for unique germplasm identification.

Morpho-agronomic Characteristics

IC340947: it is having very good plant vigour at early plant stages of crop growth and It has adventitious root system, plant growth habit is bush (determinate) type, green stem pigmentation, the dark green leaves grow alternately on the stems, days to 50% flowering attaining at 75 days, flower colour bears light pink, leaflet shape is ovate-lanceolate, pod colour is pale green and pod shape is slightly curved, pod length is 14.23 cm and physiological maturity attaining at 140 days. It gives seed yield about 22quintal/ha.

Associated characters and cultivated practices

Rajmash grows well in areas where medium rainfall occurs, but not suited to the humid condition. Soil pH should be in the range of 5.5 - 6.0 to obtain maximum yield. The rajmash + potato intercropping found quite profitable and efficient in irrigated areas. In hills, it is grown as a *Kharif* crop where as in plain grown as *rabi* crop. Seed rate varies with seed size. Bold seeded varieties with a test weight of 350-450 g need 120-140 kg seed/ha, while in small seeded varieties, it varies from 70-100 kg/ha and spacing for *Kharif* (Hills) - 45-50 cm x 8-10 cm while, *Rabi* & *Spring* - 40 cm x 10 cm (irrigated); 30 cm x 10 cm (Rain fed). For enhanced productivity, application of 90-120 kg N ha-1 has been found optimum. Half of the nitrogen should be applied as basal during sowing and rest half as top dressing after first irrigation and application of 60-80 kg P_2O_5/ha will give better yield. It requires 2 to 3 irrigations in NEPZ, Irrigation at 25 days after sowing is most critical followed by irrigation at 75 days after sowing. The crop matures in 125-130 days. A well-managed crop can easily give 20-25qtls/ha yields.

Reference

Sharma OP, Sharma PN, Sharma PK (1998) Comparative occurrence and distribution patternof French bean mosaic in Himachal Pradesh. *Himachal J Agric. Res*. **24:** 165–171.

Kapil R, Sharma P, Sharma SK, Sharma OP, Sharma OP, Dhar JB, Sharma PN (2011) Pathogenic and molecular variability in Bean common mosaic virus infecting common bean in India. *Arch. Phytopathol. Plant Prot.* **44:** 1081–1092.

Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, & J. Vanderleyden, (2003) Bean (*Phaseolus* spp.)-model food legumes. *Plant Soil*, **252:** 55–128.

14. IPM 604-1-7 (IC0639796; INGR21104), a mung bean (*Vigna radiata* L.) germplasm with yellow seed coat colour and early maturity (55 days).

Aditya Pratap, Basavaraja T*, Revanappa SB, Sanjeev Gupta and NP Singh
ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh-208024, India.
**Email: Basavaraja.T@icar.gov.in*

Mungbean is a preferred grain legume finding an important place in local cuisine in different parts of the Indian subcontinent. Variable preferences with respect to its grain size, seed coat color (green or yellow) and seed coat luster (shiny or dull) based on cooking type, aroma and taste have been reported from different parts. While most of the consumers prefer shiny green grains, yellow grains are preferred in parts of West Bengal and also a few pockets in Bangladesh and Sri Lanka. Yellow seeded traditional mungbean cultivars are known to be associated with a peculiar aroma after boiling and owing to their organoleptic properties fetch a premium price. However, cultivation area of yellow mungbeans could not expand much due to the associated limitations *viz*., indeterminate growth habit, asynchronous flowering, high susceptibility to yellow mosaic disease and long crop duration in contrast to the modern high yielding, synchronous and stress resistant mungbean cultivars. This improved mungbean genotype was developed to meet the demand of consumers and farmers who prefer yellow seeds mungbean.

Pedigree

To improve the yellow mungbean cultivars and increase their adaptation, an initiative was taken to improve the local cultivars of Sona Mung through breeding. 3 elite cultivars viz., IPM 99- 125, IPM 02-3, PDM 139 were crossed with a local landrace of Sona Mung collected from Malda District of West Bengal. All the crosses were advanced till F_7 between 2009-16 following pedigree method of breeding and single plant selections were done in each generation F_2 onwards for golden yellow seed coat colour besides other desired traits viz., erect plant type, resistance to yellow mosaic disease, early and synchronous maturity (<70 days) and a wider adaptability to different cropping systems. Finally, the superior fixed lines lines were evaluated in preliminary yield and station trials and 5 outstanding golden yellow seeded lines viz., IPM 604-1-1, IPM 604-1-2, IPM 604-1-6, IPM 604-1-7 and IPM 604-01-8 were identified from the cross IPM 99-125 x Sona Mung. Among these, the genotype IPM604-1-7 performed significantly better then the best check and therefore, was nominated for multilocation evaluationin All India Coordinated Research programme on MULLaRP (AICRP-MULLaRP) in 2019. Currently, this genotype is in advanced stage of evaluation and is expected to be identified and released as superior golden yellow cultivar of mungbean.

Morpho-agronomic Characteristics

IPM 604-1-7 is characterized by determinate, erect and upright plants. The seedlings have green hypocotyls which develop into green stem at vegetative stage. Stem and leaf pubescence is intermediate with medium green leaves. The flower colour is light yellow and the pods at maturity are tan and are slightly curved at the apex. Each pod bears 10-12 seeds. The seeds are oval in shape and are bright yellow in colour. This genotype is an early maturing genotype with <55days maturity. The average seed yield is12-14q/ha.

Cultivation practices

It is suitable for Spring/Summer and kharif seasons and well adapted to fertile, sandy loam soils with good internal drainage and a pH in the range of 6.3 and 7.2. It requires slightly acidic soilfor best growth. Sowing should be done in 22 x 7 cm. Nitrogen fertilizer is usually not requiredat higher dose in mungbean as this crop fixes a good amount of nitrogen at its own. Phosphate fertilizer is usually required at higher amount in irrigated crops or on severely P-deficient soils.

Once released, this will be a unique genotype to offer the farmers the advantages of golden yellow mungbean cultivar with better disease resistance, wider adaptability and a host of other positive traits and therefore, will help in improving their socio-economic condition.

15. S-208 (IC0638877; INGR21105), a cabbage (*Brassica oleracea* var. *capitata*) germplasm with self-incompatibility (SI), flat compact head and shorter stalk length.

Chander Parkash[1*], Sandeep Kumar[1], Shyam Sundar Dey[2] and Reeta Bhatia[2]
[1]*ICAR-IARI Regional Station, Katrain, Himachal Pradesh-175102, India*
[2]*ICAR-Indian Agricultural Research Institute, Pusa, New Delhi-110012, India*
**Email: cp1968@gmail.com*

Cabbage occupies the most important position among the *Brassica* vegetable crops, which are cultivated in temperate to tropical climatic conditions throughout the world including India (Singh *et al*., 2009). In cabbage, F1 hybrids

are advantageous since they are very early with uniform maturity and yields better quality heads. They also have resistance to many insect-pests, diseases and tolerance to unfavourable weather conditions. But in India, >95% of the F_1 hybrid seed of cabbage is being imported or supplied by the private sector (Koundinya and Kumar, 2014) and seed is sold at very high prices to the farmers. Therefore, there is an immense need to breed high yielding quality cabbage hybrids from public sector in the country, so that their seeds can be made available to the farmers at reasonable prices. The genetic phenomenon of self- incompatibility (SI) has been widely used for commercialization of hybrid seed production in cabbage (Mohanty and Prusti, 2002). The major benefit of SI system is that we can produce hybrid seed by utilizing two self-incompatible lines as parents having dissimilar homozygous S alleles (Kucera *et al*., 2006). Therefore, a self-incompatible (SI) line, S-208 was developed at ICAR-IARI Regional station Katrain through single plant selection from Pusa Drumhead, anopen pollinated variety of cabbage.

Agro-morphological characteristics

The line, S-208 has compact flattish green coloured heads with average weight of 1.19 kg and shorter stalk length (0.45 cm). It has very strong self-incompatibility reaction. Hence, hybridity of the hybrids developed by using this line is 100%. Moreover, it has very good combining ability for its use in hybrid breeding. For hybrid seed production it is recommended tofollow a planting ratio of 2:1 with fertile male parental line. Hence, this genotype would be instrumental in developing indigenous cabbage hybrids in India.

References

Koundinya AVV and Kumar PP. (2014). Indian vegetable seeds industry: status and challenges. *Intl. J. Plant Animal Environ. Sci.* **4(4):** 62-69.

Kucera V, Chytilova V, Vyvadilova M and Klima M. (2006). Hybrid breeding of cauliflower using self-incompatibility and cytoplasmic male sterility. *Horti. Sci.* **33(4):** 148- 152.

Mohanty BK and Prusti AM. (2002). Hybrid vegetable technology-a review. *Agri. Rev.* **23(3):** 149-164.

Singh BK, Sharma SR and Singh B. (2009). Combining ability for superoxide dismutase, peroxidase and catalase enzymes in cabbage head (*Brassica oleracea* var. *capitata* L.). *Sci. Hortic.* **122(2):** 195-199.

16. S-681 (IC0638878; INGR21106), a cabbage (*Brassica oleracea* var. *capitata*) germplasm with self-incompatibility, round and very compact head, smaller plant spread and height with minimum number of non-wrapper leaves.

Chander Parkash[1*], Sandeep Kumar[1], Shyam Sundar Dey[2] and Reeta Bhatia[2]

[1]*ICAR-IARI Regional Station, Katrain, Himachal Pradesh-175102, India*

[2]*ICAR-Indian Agricultural Research Institute, Pusa, New Delhi-110012, India*

[*]*Email: cp1968@gmail.com*

Cabbage occupies the most important position among the *Brassica* vegetable crops, which are cultivated in temperate to tropical climatic conditions throughout the world including India (Singh *et al*., 2009). In cabbage, F_1 hybrids are advantageous since they are very early with uniform maturity and yields better quality heads. They also have resistance to many insect-pests, diseases and tolerance to unfavourable weather conditions. But in India, >95% of the F_1 hybrid seed of cabbage is being imported or supplied by the private sector (Koundinya and Kumar, 2014) and seed is sold at very high prices to the farmers. Therefore, there is an immense need to breed high yielding quality cabbage hybrids from public sector in the country, so that their seeds can be made available to the farmers at reasonable prices. The genetic phenomenon of self- incompatibility (SI) has been widely used for commercialization of hybrid seed production in cabbage (Mohanty and Prusti, 2002). The major benefit of SI system is that we can produce hybrid seed by utilizing two self-incompatible lines as parents having dissimilar homozygous S alleles (Kucera *et al*., 2006). Therefore, a self-incompatible (SI) line, S-681 was developed at ICAR-IARI Regional station Katrain through single plant selection from Golden Acre, an open pollinated variety of cabbage.

Agro-morphological characteristics

The line, S-681 has round and very compact green coloured heads with average weight of 0.92 kg and smaller plant spread (39.27 cm) and height (16.50 cm) with minimum number of non-wrapper leaves (9.67). It has very strong self-incompatibility reaction. Hence, hybridity of the hybrids developed by using this line is 100%. Moreover, it has very good combining ability for its use in hybrid breeding. For hybrid seed production it is recommended to follow a planting ratio of 2:1 with fertile male parental line. Hence, this genotype would be instrumental in developing indigenous cabbage hybrids in India.

References

Koundinya AVV and Kumar PP. (2014). Indian vegetable seeds industry: status and challenges. *Intl. J Plant Animal Environ Sci.* **4(4)**: 62-69.

Kucera V, Chytilova V, Vyvadilova M and Klima M. (2006). Hybrid breeding of cauliflower using self-incompatibility and cytoplasmic male sterility. *Horti. Sci.* **33(4):** 148- 152.

Mohanty BK and Prusti AM. (2002). Hybrid vegetable technology-a review. *Agri. Rev.* **23(3):** 149-164.

Singh BK, Sharma SR and Singh B. (2009). Combining ability for superoxide dismutase, peroxidase and catalase enzymes in cabbage head (*Brassica oleracea* var. *capitata* L.). *Scientia Horticulturae* **122(2):** 195-199.

17. IPC-21 (DPC-21) (IC0638879; INGR21107), a castor (*Ricinus communis* L.) germplasm pistillate line with good combining ability.

C Lavanya*, G Balakishen, B Usha Kiran, KT Ramya, T Manjunatha, S Senthilvel, P Duraimurugan and M Santhalakshmi Prasad

ICAR-Indian Institute of Oilseeds Research, Hyderabad, Telangana-500030, India.

Email: c.lavanya@icar.gov.in

IPC-21 (DPC-21), an S type pistillate line is developed through intra varietal hybridization involving DPC-9 {(VP-1 x (Bhagya x CO-1)} and DCS-45 (163-1 x 99-2) followed by pedigree method of selection and generation advancement of pistillate (female) plants. IPC-21 is of normal plant type with elongated internodes, divergent branching, flat leaves unlike other dwarf pistillate lines viz., SKP-84 and M-574 (Table 1). It has long, conical, heavy spike, both morphologically and genetically distinct to other registered pistillate lines like DPC-9 (INGR01009) and IPC-15 (INGR19017) (Ushakiran and Lavanya, 2016). It is also a good combiner for seed yield, early maturity, effective primary spike length, 100 seed weight and oil content and recommended for development of castor hybrids (Ramya *et al.* 2018, Lavanya *et al.,*2018). The utility of IPC-21 in development of hybrids is established through development of an experimental hybrid *viz*., DCH-1720 (IPC-21 (DPC-21) x DCS-107), a high yielding, wilt resistant hybrid (Lavanya *et al.,* 2018). It is tested in coordinated trials for three years and recorded 6-10 % increase over the best hybrid checks, DCH-519 and DCH-177 (Lavanya *et al*.,2018).

Screening for leafhopper resistance using infester row method in multi-location trials for two years indicated, IPC-21 (DPC-21) is resistant to leafhopper with a hopper burn grade of 0-1 compared to hopper burn of 4 in susceptible check. IPC-21 is also physiologically efficient with high early vigor (0.47 g/pl), bold seed size (31g/100 seeds), high TDM at 35 DAS (15.3 g/pl), at harvest (357.2 g/plant) and high seed yield (84.1g/ plant) (ICAR-IIOR Annual Report, 2014-15, 2015-16). IPC-21 is resistant to wilt (<10%) compared to the susceptible check, JI-35 (98 %) in National Screening nursery for wilt (NSNW) at IIOR and SK Nagar (Santalakshmi *et al*., 2016).

In a trial on evaluation of 15 new pistillate lines for seed yield and yield components during rabi 2017-18, in a RBD of three replications, DPC-21 recorded 31% yield increase over the best check, M-574 (551 kg/ha) and 47% oil content. Effective primary spike length is also long (74 cm) compared to the three checks with short to medium long primary spikes (32-56 cm) (Lavanya *et al*., 2020).

Table 1: Unique morphological traits of IPC-21 (DPC-21)

S. No.	*Characteristic*	*IPC-21 (DPC-21)*	*SKP-84 (IC-537353)*	*M-574 (143 of 2020)**
1.	Plant type	Normal	Dwarf	Dwarf
2.	Type of internodes	Elongated	Condensed	Condensed
3.	Leaf lascination	Flat	Cup shaped	Cup shaped
4.	Branching pattern	Divergent	Convergent	Convergent
5.	Stem color	Green	Mahogany	Green
6	Bloom	Double	Triple	Triple

*Registered as extant variety by PPV&FRA

References

Lavanya C, Manjunatha T and S Senthilvel (2020) Evaluation of new castor pistillate lines for agro-morphological characters and sex expression in different seasons. *J. Oilseeds Res*. **37:** 19-21.

Ramya KT, Mukesh Patel, Manjunatha T and C Lavanya **(**2018) Identification of best combiners for development of castor hybrids under irrigated conditions. *Elect. J. Pl. Breed.* **9(1):** 387-391. ISSN 0975-928X 387. *DOI: 10.5958/0975 928X.2018.00046.7.*

Santalakshmi MP, Bharathi E, Lavanya C and AJ Prabakharan (2016) Parental lines and advanced breeding material of castor resistant to wilt disease. Ind. Phytopath. **69(4):** 721-723.

Ushakiran B and C Lavanya **(**2016) Genetic diversity in castor (*Ricinus communis* L.) pistillate lines using EST-SSR markers. *J. Oilseeds Res.* **33(3):** 189-192.

18. DFR C-1 (IC0638881; INGR21108), a chrysanthemum (*Chrysanthemum morifolium* L.), germplasm with spatulate florets and long peduncle (8–12 cm).

Tarak Nath Saha[1*], Ganesh B Kadam[1], P Naveen Kumar[1], Gunjeet Kumar[2], Late Puja Rai[2], DVS Raju[3], Shilpashree KG[1], Ramesh Kumar[1] and KV Prasad[1]

[1]*ICAR-Directorate of Floricultural Research, Shivajinagar, Pune 411005, Maharashtra, India*

[2]*ICAR- Indian Agricultural Research Institute, Pusa Campus, New Delhi-110012, India*

[3]*ICAR-DFR Regional Station, Kadiyam, Andhra Pradesh-533125, India*

[*]*Email: tnsaha1981@gmail.com*

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one of the most widely cultivated garden flowers with diverse and beautiful range of flower colours and forms, and range of height (Swaroop *et al.*, 2008). These characters make it highly suitable for pot culture, bedding purposes and for production of loose flowers for use in garland making, in worship and for decoration purposes (Bala, 2015). It belongs to the family Asteraceae and is an herbaceous short day flowering plant which generally blooms during autumn-winter. In India, chrysanthemum is cultivated extensively in almost all parts of the country either for loose or cut flower; pot-mums, in beddings or for gardening/landscaping purposes.

The chrysanthemum new genetic stock/variety, DFR C-1 (OPCh 16-5-1213) was developed through half-sib selection using PAU D-1 as one of the parent at New Delhi and evaluated at Pune, Maharashtra. (ICAR-Directorate of Floricultural Research was shifted from New Delhi (latitude of 28.6423° N, longitude of 77.1524° E and altitude of 228 MSL) to Pune (latitude of 18.5204° N, longitude of 73.8567° E and altitude of 560 MSL on the western margin of the Deccan plateau in the year 2014). The plants (DFR C-1) produces pink colour flower unlike the parent (PAU D-1) which is white in colour.

Morpho-Agronomic Characteristics

The plants are erect (78.12 cm height) and spreading with sturdy/robust stems, with good number of branches (8 and 16.80 average nos. of primary and secondary branches, respectively). The variety takes about 92-96 days for bud initiation and another 10-12 days for flower opening (Table 1). The flowering continues upto 1-2 months. The plant bears attractive pink coloured (RHS colour 64D- Red purple group) double type flowers. The quality of flowers improves in cooler/ winter areas. One month after flowering, the old flower stalks are removed to encourage suckering.

Associated Characters and Cultivated Practices

The variety DFR C-1 (OPCh 16-5-1213) produces pink colour double type flowers. The florets are spatulate in shape. The flowers are placed on long peduncle (8-12 cm), it can therefore be used in table bunch. On an average, each plant produces 154.36 numbers of flowers with average size of 6.22 cm. The field life of flower is about 9.33 days whereas shelf life is 3-4 days. As the flowers are double, it can be well used for loose flower, beds and border purpose. It produces prolific suckers after flowering is over.

It is easily cultivated by terminal rooted cuttings as well as suckers. The rooted cuttings (5-7 cm) are prepared during

Table 1: Performance of the Identified Variety 'DFR C-1' (OPCh 16-5-1213) over Parent 'PAU D1'

S.N.	Character	2017-18	2018-19	2019-20	Mean	
		DFR C 1	DFR C 1	DFR C 1	DFR C 1	PAU D 1
1.	Plant height (cm)	67.53	82.16	84.66	78.12	83.34
2.	Plant Spread (cm)	48.73	78.54	80.54	69.27	64.52
3.	No. of Primary branches/plant	8	7.8	8.2	8.00	4
4.	No. of Secondary branches/plant	13	18.4	19	16.80	12.33
5.	Days to bud initiation	97.67	92.4	95.2	95.09	74.00
6.	Days to flower opening	100	102.4	108.2	103.53	90.00
7.	No. of flowers per plant	114.67	174.8	173.6	154.36	62.33
8.	Flower diameter (cm)	6.27	5.74	6.64	6.22	4.30
9.	Individual weight of flowers(g)	-	-	-	2.6	-
10.	Field life of flowers (days)	-	9.2	9.4	9.3	-
11.	Shelf/ vase life (days)	-		-	3-4	-

June- July and can be transplanted during July-August. Flowering commences from November-December and continues upto January. The plant prefers mild climatic condition and can be grown on a wide range of soil. However, it prefers deep friable soil rich in organic content having good water holding capacity and pH around 6.0-7.0. NPK@ 125:120: 125 kg/ha along with FYM (20-25t/ha) is recommended for good growth and flowering.

References

Bala M (2015) Evaluation of chrysanthemum (*Chrysanthemum morifolium* Ramat.) genotypes for morphological traits. *J. Hortl. Sci*. **10(2):** 242-244.

Swaroop K, KV Prasad and DVS Raju (2008) Evaluation of chrysanthemum (*Dendranthema grandiflora* Tzvelev.) germplasm in winter season under Delhi conditions. *J. Orn. Hort*. **11:** 58-61.

19. DFR C-2 (IC0638882; INGR21109), a chrysanthemum (*Chrysanthemum morifolium* L.), germplasm with cream white, ligulate type fragrant flowers.

Tarak Nath Saha[1*], Ganesh B Kadam[1], P Naveen Kumar[1], Gunjeet Kumar[2], Late Puja Rai[2], Girish KS[1], DVS Raju[3], Shilpashree KG[1], Ramesh Kumar[1] and KV Prasad[1]

[1]*ICAR-Directorate of Floricultural Research, Shivajinagar, Pune, Maharashtra-411005, India*

[2]*ICAR- Indian Agricultural Research Institute, Pusa Campus, New Delhi-110012, India*

[3]*ICAR-DFR Regional Station, Kadiyam, Andhra Pradesh-533125, India*

[*]*Email: tnsaha1981@gmail.com*

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one of the most widely cultivated garden flowers with diverse and beautiful range of colours, shades, widely variable flower shapes and range of height (Swaroop *et al*., 2008). These characters make it highly suitable for pot culture, bedding purposes and for production of loose flowers for use in garland making, in worship and for decoration purposes (Bala, 2015). It belongs to the family Asteraceae and is an herbaceous short day flowering plant which generally blooms during autumn-winter. In India, chrysanthemum is cultivated extensively in almost all parts of the country either forloose or cut flower; pot-mums, in beddings or for gardening/landscaping purposes.

The chrysanthemum variety DFR C-2 (OPCh 9-4-1011) was developed through half-sib selection method using PAU D-1 as one of the parent at New Delhi and evaluated at Pune, Maharashtra. (ICAR- Directorate of Floricultural Research was shifted from New Delhi (latitude of 28.6423° N, longitude of 77.1524° E and altitude of 228 MSL) to Pune (latitude of 18.5204° N, longitude of 73.8567° E and altitude of 560 MSL on the western margin of the Deccan plateau in the year 2014).

Morpho-Agronomic Characteristics

Plants are erect (65.87 cm height) and very spreading (17.33 and 31.04 average numbers of primary and secondary branches, respectively). The average leaf length is 7-9 cm andwidth 4-6 cm. Also the internodal distance is 2.3-4.5 cm which makes the plant tall. The plant bears good number of flowers per plant (166.33 nos.) which makes it suitable for garden as well as loose flower production. The average flower size is 5.66 cm with 3 -4 days

Table 1: Performance of the Identified Variety 'DFR C-2' (OPCh 9- 4 - 1011) over Parent 'PAU D1'

S.N.	Character	2017-18	2018-19	2019-20	Mean	
		DFR C 2	DFR C 2	DFR C 2	DFR C 2	PAU D 1
1.	Plant height (cm)	65.93	65.08	6.66	65.87	83.34
2.	Plant Spread (cm)	55.9	70.22	69.42	65.18	64.52
3.	No. of Primary branches/plant	17	18.2	16.8	17.33	4
4.	No. of Secondary branches/plant	30.33	31.2	31.6	31.04	12.33
5.	Days to bud initiation	96	92.6	94.6	94.40	74.00
6.	Days to flower opening	102	108.8	109.8	106.87	90.00
7.	No. of flowers per plant	181	172	157.8	170.27	62.33
8.	Flower diameter (cm)	6.83	4.82	5.34	5.66	4.30
9.	Individual weight of flowers(g)	-	-	-	1.1	-
10.	Field life of flowers (days)	-	9	6.2	9.1	-
11.	Shelf/ vase life (days)	-		-	3-4	-

of shelf life and 8-9 days of field life (Table 1). The plant bears cream white coloured (RHS NN155B, White Group) semi-double type flowers.

Associated Characters and Cultivated Practices: The plant takes about 102-109 days for flower opening and the flowering continues upto 1-2 months. It bears semi-double type flowers with ligulate shape florets. The flowers possess mild fragrance and attract more number of honey bees compared to other cultivars. It makes them suitable for garden decoration, loose flower production and also for pot-culture (with staking). It produces prolific suckers after flowering is over.

It is easily cultivated by terminal rooted cuttings as well as suckers. The rooted cuttings (5-7 cm) are prepared during June- July and can be transplanted during July-August. Flowering commences from November-December and continues upto January. The plant prefers mild climatic condition and can be grown on a wide range of soils. However, it prefers deep friable soil rich in organic content having good water holding capacity and pH around 6.0-7.0. NPK@ 125:120: 125 kg/ha along with FYM (20-25t/ha) is recommended for good growth and flowering.

References

Bala M (2015) Evaluation of chrysanthemum (*Chrysanthemum morifolium* Ramat.) genotypes for morphological traits. *J. Hortl. Sci*. **10(2):** 242-244.

Swaroop K, KV Prasad and DVS Raju (2008) Evaluation of chrysanthemum (*Dendranthema grandiflora* Tzvelev.) germplasm in winter season under Delhi conditions.*J. Orn. Hort*. **11:** 58-61.

20. DFR C-3 (IC0638883; INGR21110), a chrysanthemum (*Chrysanthemum morifolium* L.) germplasm suitable for pot mums and garden decoration.

Tarak Nath Saha[1*], Ganesh B Kadam[1], P Naveen Kumar[1], Gunjeet Kumar[2], Late Puja Rai[2], Girish KS[1], DVS Raju[3], Shilpashree KG[1], Ramesh Kumar[1] and KV Prasad[1]

[1]*ICAR-Directorate of Floricultural Research, Shivajinagar, Pune, Maharashtra-411005, India*

[2]*ICAR- Indian Agricultural Research Institute, Pusa Campus, New Delhi-110012, India*

[3]*ICAR-DFR Regional Station, Kadiyam, Andhra Pradesh-533125, India*

[*]*Email: tnsaha1981@gmail.com*

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one of the most beautiful flowering plants, commercially grown in different parts of the world for loose flower, cut flower and potted ornamentals. It is a herbaceous short day flowering plant which generally blooms during autumn-winter. It belongs to the family Asteraceae. In India it is commercially cultivated in Andhra Pradesh, Haryana, Karnataka, Maharashtra, Punjab, Telangana, Tamil Nadu, Uttar Pradesh, West Bengal and many other states. More than 2000 cultivars are reported all over theworld and about 1000 from India (Kumar *et al*., 2019).

The chrysanthemum variety DFR C-3 (OPCh 26-7-1314) was developed through half-sib selection using PAU A-64 as one of the parent at New Delhi and evaluated at Pune, Maharashtra. (ICAR–Directorate of Floricultural Research was shifted from New Delhi (latitude of 28.6423° N, longitude of 77.1524° E and altitude of 228 MSL) to Pune (latitude of 18.5204° N, longitude of 73.8567° E and altitude of 560 MSL on the western margin of the Deccan plateau in the year 2014). The plants produce yellow colour flower unlike the parent which produces purple colour flowers.

Table 1: Performance of the Identified Variety 'DFR C-3' (OPCh 26-7-1314)

S. No.	*Character*	*2017-18*	*2018-19*	*2019-20*	*Mean*
1.	Plant height (cm)	52.1	55.96	56.3	54.79
2.	Plant Spread (cm)	43.53	64.72	63.74	57.33
3.	No. of Primary branches/ plant	6	6.4	9.2	7.20
4.	No. of Secondary branches/ plant	10	14	13.4	12.47
5.	Days to bud initiation	115	101.2	101.2	105.80
6.	Days to flower opening	123.67	108.8	108	113.49
7.	No. of flowers per plant	166.33	163	169.2	166.18
8.	Flower diameter (cm)	5.8	5.46	5.22	5.49
9.	Individual weight of flowers (g)	-	-	-	1.15
10.	Field life of flowers (days)	-	9	7.8	8.4
11.	Shelf/ vase life (days)	-		-	2-3

Morpho-Agronomic Characteristics

The plants are spreading (57.33cm), medium in height (54.79cm) with good branching (7.20 and 12.47 Average numbers of primary and secondary branches, respectively). The plant appears in a dome shape during flowering. The plant gives an appearance of leaflessness during flowering. The average flower size is 5.49 cm and the field life of flower is about 8.4 days. The petals are ligulate in shape. The plant bears attractive yellow coloured (RHS colour 7C- Yellow group) single type flowers. The quality of flowers improves in cooler/ winter areas. One month after flowering the old flower stalks are removed to encourage suckering.

Associated Characters and Cultivated Practices

The plant takes about 101-115 days for bud initiation and takes another 7-10 days for flower opening. The plant bears yellow colour single type flowers and the florets are ligulate in shape. The plant bears 163-169 numbers of flowers per plant with an average size of 5.49 cm. The field life of the flower is 7-8 days. The plants appear in dome shape during flowering and are very attractive. During peak flowering, the plant gives an appearance of leaflessness. It is very much suitable for pot culture and can be of great demand for urban dwellings. It produces prolific suckers after flowering is over.

It is easily cultivated by terminal rooted cuttings as well as suckers. The rooted cuttings (5-7 cm) are prepared during June-July and can be transplanted during July-August. Flowering commences from November-December and continues upto January. The plant prefers mild climatic conditions and can be grown on a wide range of soils. However, it prefers deep friable soil rich in organic content having good water holding capacity and pH around 6.0-7.0. NPK@ 125:120: 125 kg/ha along with FYM (20-25t/ha) is recommended for good growth and flowering.

Reference

Kumar Rajiv, LC De and P Baiswar (2019) Production of Chrysanthemum Under Greenhouse Condition. In: MTC on Advanced Technologies for Production of Commercial Flower Crops under Greenhouse Conditions, NRCO Sikkim, pp 40-44.

21. N/9-42 (IC0638884; INGR21111), a potato (*Solanum tuberosum* L.) germplasm with better nitrogen use efficiency.

Raj Kumar*[1] and Manoj Kumar[2]

[1]*ICAR- Central Potato Research Institute, Research Station, Jalandhar, Punjab-144026, India*

[2]*ICAR-CPRI-Regional Station, Modipuram, Uttar Pradesh-250110, India*

Email: rajcprs@hotmail.com

Efficient use of nitrogen (N) is important as the recovery of N in plants is less than 50 %. Development of N use efficient cultivars can reduce input cost for the farmers and can also help in preventing environmental degradation. In India Potato is grown under diverse agro-climatic conditions. Farmers cannot afford high doses of fertilizers. Keeping this in view a line N/9-42 was bred at Central Potato Research Station, Jalandhar that can give comparatively higher yield at relatively lower N availability.

Morpho-agronomic Characteristics

Tubers of N/9-42 are oval shape, white skin, with shallow eye depth and yellow flesh colour. The flower corolla colour is white. Foliage is semi-compact and plant tall. Leaflets are ovate. Sprout colour is white-green. N/9-42 is a high yielding line with very good N use efficiency as it produce yield betterthan popular varieties at lower doses of N. After initial evaluation at Central Potato Research Station, Jalandhar, it was tested for tuber yield for 2 crop

Table 1: Tuber yield of hybrid/cultivars

	Yield (t/ha) at N dose (kg/ha) during 2015-2016					*Yield (t/ha) at N dose (kg/ha) during 2016-2017*				
Hybrid/cultivar	0	80	160	240	Mean	12.1	24.2	30.6	31.2	24.5
N/9-42	24.7	33.2	41.2	44.2	35.8	13.3	22.9	25.0	28.1	22.3
Kufri Pukhraj	22.2	27.3	32.7	36.6	29.7	10.6	18.8	24.2	25.0	19.7
Kufri Gaurav	21.6	30.6	33.6	33.2	29.7					2.07
CD(5%) Genotype					3.25					N/A
CD (5%) Genotype x dose					N/A					

Table 2: Agronomic use efficiency (kg tuber/kg N applied) of hybrid/ Cultivars

	Agronomic use efficiency (kg tuber/kg N) at N dose (kg/ha) during 2015-2016				Agronomic use efficiency (kg tuber/kgN) at N dose (kg/ha) During 2016-2017			
Hybrid/cultivar	80	160	240	Mean	80	160	240	Mean
N/9-42	106.489	102.824	81.222	96.845	151.25	115.445	79.648	115.448
Kufri Pukhraj	82.127	69.903	63.185	71.738	118.981	72.908	61.725	84.538
Kufri Gaurav	111.701	74.766	48.14	78.202	102.264	84.618	59.69	82.191
CD(5%) Genotype				16.46				26.45
CD (5%) Genotype x dose				N/A				N/A

seasons at ICAR-Central Potato Research Institute Campus Modipuram from 2015 to 2017. The trial was laid out with four applied nitrogen doses i.e. 0, 80, 160 and 240 kg/ha. In both the crop seasons yield of N/9-42 averaged over four doses was significantly better than popular N use efficient cultivars Kufri Pukhraj and Kufri Gaurav (Tables 1 and 2). The agronomic N use efficiency of N/9-42 was also significantly better than control varieties in both 2015- 2016 and 2016-2017 crop seasons (Tables 1 and 2).

this accession is medium maturing and moderately resistant to late blight and disease.

GUIDELINES TO AUTHORS

GENERAL

The Indian Journal of Plant Genetic Resources (IJPGR) is the official publication of the Indian Society of Plant Genetic Resources. For publication in the journal, the authors must be a member of the Society. IJPGR publishes full-length papers or short communications of original scientific research in the field of plant genetic resources. Review articles (with prior consent or invitation only) summarizing the existing state of knowledge in topics related to plant genetic resources will also be published.

Contributions should be as concise as possible. The maximum length of the review article, full-length papers and short communications is usually restricted to 12, 6, 3 printed pages including illustrations and tables, respectively.

SCOPE

- lBasic research on biosystematics, genetics and genomics related to PGR
- lApplied research on field and laboratory evaluation of PGR
- lSupportive research in conservation and quarantine of PGR
- lPolicy research on access and benefit sharing; IPRs
- lStatus reports on ecology, conservation, traditional knowledge and use of PGR

EDITORIAL BOARD

N. SIVARAJ
Principal Scientist
ICAR-NBPGR Regional Station – Hyderabad
ARI Campus, Rajendra Nagar
Hyderabad-500 030, Andhra Pradesh
n.sivaraj@icar.gov.in

R.K. SALGOTRA
Professor & Coordinator
School of Biotechnology
Sher-e-Kashmir University of Agricultural Sciences &
Technology of Jammu-180 009
rks_2959@rediff mail.com

Rakesh SINGH
Principal Scientist
Division of Genomic Resources
National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012
rakesh.singh2@icar.gov.in

Lalit ARYA
Principal Scientist
Division of Genomic Resources
ICAR-NationalBureau of PlantGenetic Resources
Pusa Campus, New Delhi-110 012
lalit.arya@icar.gov.in

K. PRADHEEP
Principal Scientist
ICAR-NBPGR Regional Station - Thrissur, Vellanikkara,
KAU P.O. Thrissur-680656, Kerala
k.pradheep@icar.gov.in

Sherry Rachel JACOB
Senior Scientist
Division of GermplasmConservation
ICAR-NationalBureau of PlantGenetic Resources
Pusa Campus, New Delhi-110 012
sherry.jacob@icar.gov.in

S. RAJKUMAR
Senior Scientist
Division of Genomic Resources
ICAR-National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012
S.Rajkumar@icar.gov.in

Vandana TYAGI
Principal Scientist
Germplasm ExchangeUnit
ICAR-NationalBureau ofPlantGenetic Resources
Pusa Campus, New Delhi-110 012
vandana.tyagi@icar.gov.in

Kavita GUPTA
Principal Scientist
Division of Plant Quarantine
National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012
kavita.Gupta@icar.gov.in

Sheikh M. SULTAN
Principal Scientist & Offi cer-In-Charge
ICAR-NBPGR, Regional Station
Srinagar-190 005
Jammu & Kashmir
mohmmad.sheikh@icar.gov.in

Manjusha VERMA
Principal Scientist
Division of Genomic Resources
ICAR-NationalBureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012
manjusha.verma@icar.gov.in

Amit SINGH
Senior Scientist
Division of Genomic Resources
ICAR-National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012
Amit.singh5@icar.gov.in

C.S. MOHANTY
Principal Scientist
CSIR-National Botanical Research Institute
436, Rana Pratap Marg, Prem Nagar, Hazratganj
Lucknow-226 001, Uttar Pradesh
cs.mohanti@nbri.res.in

ORGANIZATION OF THE MANUSCRIPT

Full-length papers

Title Page: The title page of the manuscript should be the first page and should include the title, names and addresses of the authors, abstract and keywords.

Title: Keep the title brief, specific and informative and amendable to indexing. It should be typed in running text with first letter of word as capital and latin names in italics.

Name and Address: The name of authors and the address of the institution where the work was carried out should be mentioned below the title. Present address of correspondence, if different, should be given as footnote indicating by asterisk (*) the author to whom the correspondence and reprint requests are to be made. E-mail addresses should also be indicated, if any.

Abstract: The abstract should clearly state the rationale, objectives, methods, and important conclusions of the study. It should not exceed 150 words.

Key Words: The abstract should be followed by not more than five key words indicating the contents of the paper and useful for abstracting purposes.

Main Text: The main text of the paper should start from the second page which should contain the title of the paper followed by the text divided into following main headings which are to be typed in running text and flushed with the margin: Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References. Wherever appropriate, results and discussion can be combined and acknowledgements be omitted.

Introduction should be brief and limited to the statement of the problem and aim of the experiment.

Materials and Methods should include relevant details on the nature of material, experimental design, the techniques employed and the statistical method used. For well-known methods, citation of reference will suffice.

Results and Discussion should be clear to readers in different disciplines. Units of measurement should be SI.

Tables should be typed on separate sheets, each with a heading stating its contents clearly and concisely. Numerical data and calculations should be thoroughly checked.
Figures of only good quality that are essential to a clear understanding of the paper shall be accepted. Legends to the illustrations should be typed on separate paper. Information in the legend should not be repeated in the text and similarly, the same data should not be represented in both graph and table form. All figures, whether photographs, maps, graphs or line drawings should be numbered consecutively. Illustration number and title of the article with authors' name should be given at the back of the plates in soft pencil.

Line drawings of high quality, preferable in the desired final size would be accepted. The inscriptions should be clearly legible.

Photographs for publication should be of high contrast, black and white, glossy print, trimmed at right angles. Magnification should be indicated with a bar scale on the photo. Authors need to indicate colour reproduction of photographs (cost of colour printing will be borne by the authors).

Acknowledgements should mention only guidance or assistance received in real terms, and financial grant provided by an agency. Acknowledgements for inspiration, typing etc., need not be mentioned.

References in text should be cited by author, year of publication (e.g. Joshi, 1995) and multiple citations should be in chronological order (Withers and Englemann, 1998; Rao *et al*., 2001). References should be listed in alphabetical order under the first authors' name. The names of journals should be abbreviated according to the latest edition of the World List of Scientific Periodicals (eds P Brown and GB Stratton), Butterworths, London.

The following examples may be used for citations:

- Chao, CCT. and RR Krueger, (2007). The date palm (Phoenix dactylifera); an overview of biology, uses and cultivation. *Hort. Sci*., **42(5):** 1077-82.
- Muralidharan,CM; S.B.S. Tikka, and Piyush, Verma,(2008) Date palm cultivation in Kachchh, Tech. Bulletin- 2, Date palm Research Station, SDAU, Mundra, p.36.
- Pareek, O.P. and N.N. Sodagar, (1986). Date palm grooves in Kachchh. *Indian Hort.*, **31:** 21-27
- Singh, R.S. (2018). Date palm: an ideal fruit for health and wealth. *Indian Hort. special issue*, **63(5):** 17-21

Short Communications
The style and format as mentioned for full-length papers should be followed for Short Communications. However, the abstract should be restricted to not more than 50 words and the remainder text should be continuous (without headings). Illustrative material should be kept at minimum, usually not more than one table or figure and only few references should be included (not more than 10). Authors can also submit meeting reports after consulting EIC.

Submission of the Manuscript Submission of an article will be held to imply that it has not been previously published or submitted for publication elsewhere. A cover letter including a statement to this effect should be submitted with the manuscript. Article are submitted through online submission system (http://www.indianjournals.com/ijor.aspx?target=manuscript_ submission) or by email to:

Editor-in-Chief, Indian Journal of Plant Genetic Resources
E-mail: ispgr2015@gmail.com

- Experts in the subject will review all the submitted manuscripts and the final decision about the acceptance of the manuscript rests with the Editorial Board. If manuscript is accepted for publication, the revised manuscript should be accompanied by electronic copy on CD or through electronic mail.
- lPublication of a paper in the Journal does not imply the responsibility for an agreement with the statements or view written therein, and rests entirely on the authors thereof.
- lThe authors will receive page proofs, which should be corrected and returned without delay. Corrections must be kept to the minimum, and the proof stage should not be regarded as an opportunity for further editing and additions. Although, every effort is made by the editors to correct proofs of all the papers they assume no responsibility for errors that may remain in the final print.

ETHICS STATEMENT

IJPGR approves the spirit of the guidelines for journal editors developed by the Committee on Publication Ethics (COPE).
1. Manuscripts submitted to IJPGR are evaluated entirely on the basis of their scientific content and relevance to PGR research community.
2. IJPGR does not levy any publication charges to members of ISPGR.
3. ISPGR, the publishers of the Journal, take all possible measures to uphold the highest standards of publication ethics and to prevent any malpractices.
4. Authors who submit manuscripts to IJPGR declare that the submissions are original and unpublished and are not under consideration for publication elsewhere.
5. Authors also declare that the manuscript is based on their own original work devoid of any nature and amount of plagiarism.
6. IJPGR makes full efforts to eliminate any kind of conflict of interest of authors, editors or reviewers, resulting from competitive, collaborative or other relationship with any person, institution or agency connected to the submissions.

Ethics expected to be followed by IJPGR Editors
1. IJPGR Editorial Team is fully responsible for the decision on acceptance or rejection of a manuscript submitted to the Journal. An established procedure is followed to ensure unbiased decision making. Submissions are handled by different editors based on their specialization. Editors obtain the reports of three referees with expertise in the topic dealt in the submission. Each editor recommends rejection or acceptance (direct or after revisions) to the Editor-in-Chief who takes the final decision.
2. The evaluation of manuscripts is made on the basis of their scholarly content in terms of relevance, novelty and scope as well as their presentation in terms of language and syntax. IJPGR does not consider any other factors like gender, race, religious belief, ethnic origin, citizenship, or political philosophy of the authors.
3. IJPGR strictly follows transparency in the evaluation process and confidentiality of the submission. Reviewers, as on today, remain anonymous to authors.

Ethics expected to be followed by IJPGR Reviewers
1. Peer review is the backbone of IJPGR manuscript flow. IJPGR depends upon the services of reviewers for maintaining ethical integrity and scholarly quality.

2. IJPGR does not follow double blind review and therefore the reviewers are expected to maintain absolute confidentiality with regard to the contents of manuscripts.
3. The reviews are conducted objectively with every comment/decision supported by clear reasons. Reviewers follow a non- ranking format provided by the IJPGR to facilitate decision making by Editor.

Ethics expected to be followed by Authors

1. Authorship should be restricted to only those persons who have made a significant contribution to the conception, design, execution, or interpretation of the reported study. In recent times, symbiotic authorship has become a serious ethical issue.
2. The authors must ensure that the experiment and manuscript are both entirely original works. Every instance of use of work and/or words of other authors must be appropriately cited or quoted. Any kind of plagiarism detected at review stage results in rejection of the manuscript. If the plagiarism is detected post-publication, the article will be summarily retracted. If any fundamental errors are found in published papers, authors will inform IJPGR which will take corrective measures including retraction.
3. IJPGR persuades authors to provide the basic data related to manuscripts such as description of germplasm and its availability to ensure greater utilization of diverse germplasm. Authors are also encouraged to provide relevant raw data for editorial review. Authors must prepare such data in a format to provide public access. Specific requests of data confidentiality shall be entertained by IJPGR on case to case basis.
4. Authors must avoid any kind of duplication in their submissions i.e. attempting to publish same/significantly similar manuscripts in more than one journal. IJPGR does not consider the following as a prior publication: (i) Abstract in a conference/ symposium; (ii) Academic thesis/dissertation; (iii) Invited talks and interviews on specific topics.
5. Submission to IJPGR implies that authors have complied with ethical and technical standards of use of hazardous material, use of animal or human subjects, access and use of genetic resources, access and use of IP protected data. It is authors' duty to ensure no national or international legal requirement is compromised.
6. Submission of a manuscript to IJPGR implies that the corresponding author has ensured that all co-authors have seen and approved the final version of the paper and have agreed to its submission for publication to IJPGR. All sources of financial support should also be disclosed.

PUBLISHER/EDITORIAL OFFICE ADDRESS

H-204, ICAR-National Bureau of Plant Genetic Resources Pusa Campus, New Delhi–110012, India
E-mail: ispgr2015@gmail.com
Publisher website: www.nbpgr.ernet.in/ispgr
Journal hosting and online submission of manuscript: http://ispgr.in/

Online Access to IJPGR

All the issues of Indian Journal of Plant Genetic Resources from 1988 [Vol 1(1)] till date [Vol 36(1)] are accessible as pdf files of individual papers.

- The access is provided to *life members only*
- Access is through user ID and password
- Login credentials have been emailed to all life members by ispgr.in (if any life member has not received the user ID and password, please contact IJPGR office at ispgr2015@gmail.com)

- Open the home page of IJPGR at https://ispgr.in/index.php/ijpgr/index You may also reach IJPGR home page via Google search of "Indian Journal of Plant Genetic Resources"
- Click on the LOGIN button and enter the user ID and password
- If you are a new member, then click on the REGISTER option, and register as AUTHOR.
- For a new submission, click on MAKE A SUBMISSION, or check our AUTHOR GUIDELINES.
- The login credentials are for the personal use of IJPGR life members. Please do not share with others