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RESEARCH ARTICLE

Study of Maternal Influence and Identification of Stable Donors for Grain Protein Content in Rice (*Oryza sativa* L.)

G Kiruba[1] and S Geetha[1*]

Abstract

An experiment was conducted during *kharif* 2021 at Paddy Breeding Station (PBS), Coimbatore, Regional Research Station (RRS), Paiyur and Tamil Nadu Rice Research Institute (TRRI), Aduthurai; and during *kharif* 2022 at Paddy Breeding Station (PBS), Coimbatore, to identify the stable donors and to determine the existence of maternal influence for grain protein content. Genetic diversity studies using a panel of 169 rice genotypes, including landraces and cultivated varieties, revealed significant variation in grain protein content. The genotypes IG74, Gandhasala, Ponkambi samba, Purple puttu and Burma kavuni were found to be promising and stable genotypes with higher grain protein content (>10%) across three locations. The genotypes IG74 and Gandhasala were found to be the best parents for the breeding programme to enhance grain protein content together with grain yield. The maternal influence on grain protein content was observed, which indicated that high protein donors could be recommended to be used as female parents.

Keywords: Combining ability, D^2 analysis, Maternal influence, Protein content, Rice, Stability

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Introduction

Rice (*Oryza sativa* L.) is the sole crop among cereals containing a storage protein called "Oryzenin" which have more balanced amino-acid profile when compared to the prolamin-rich storage protein. Rice grain protein content (GPC) plays a crucial role in determining the cooking, nutritional and eating quality (Chen *et al*., 2023). The protein components of rice are generally considered hypoallergenic and are suggested that the rice proteins might exert several health benefits with their anti-hypertensive, anti-oxidative, anti-cancer and anti-obesity activities (Amagliani *et al*., 2019). Approximately one-third of the global population is currently suffering from protein malnutrition. Though rice usually has the lowest grain protein content among cereal grains, the net protein utilization from rice is higher than that of the other cereal grains (Juliano, 1992). ICAR-NRRI developed India's first high-protein rice varieties, CR Dhan 310 (10.3% protein) and CR Dhan 311 (Mukul; ~10.1%), through classical backcross breeding in 2015–2016, demonstrating that high protein content can be combined with high grain yield (4.5 and 4.3 t/ha, respectively) (Mahender *et al.,* 2016). Both these high-protein varieties CRDhan 310 and CRDhan 311, were found to contain QTL (*qGPC1.1*) on chromosome 1.

The unique germplasm collections in India have several potential genes quite obvious to substantiate the nutritional needs of the global population. Hybridization among such diverse germplasm accessions helps to generate desirable recombinants for the rice improvement programme. A successful breeding programme requires knowledge of gene interactions, selection strategies, identifying the donor parents and choosing optimal parent combinations. Diversity could be well depicted by D^2 and Principal Component Analysis, which play a crucial role in the selection of parents for hybridization programme. Among several mating designs, Griffing's full diallel analysis involving the reciprocal crosses play unique role in determining the existence of maternal effect. Keeping these points in view, a study was taken up to improve grain protein content in rice through the selection of parents based on stability, PCA and D^2 analysis and development of F_1s to study the gene action and maternal influence on grain protein content.

Materials and Methods

To assess the G×E interaction on protein content, a panel containing 169 rice genotypes, which included landraces, improved lines and cultivated varieties were raised in an alpha lattice design, consisting of 13 blocks. Each genotype was raised in two rows with two replications adopting a spacing of 50 cm × 30 cm between the rows and plants with a single seedling per hill in each replication in three different environments *viz*., Paddy Breeding Station (PBS), Coimbatore, Regional Research Station (RRS), and Paiyur and Tamil Nadu Rice Research Institute (TRRI), Aduthurai, during *kharif* season of 2021 (Table 1). The experimental material comprises landraces, improved lines and cultivated varieties of different origins. The following parameters were studied: DFF - days to 50% flowering (days), PH - plant height (cm), NPT - number of productive tillers (no.), PL - panicle length (cm), FLL - flag leaf length (cm), FLW - flag leaf width (cm), NFG - number of filled grains per panicle (no.), TGW - 1000-grain weight (g), SPY - single plant yield (g) and PC - protein content (%).

Grain protein content was estimated using high-throughput Near Infra-Red Spectroscopy (NIR; ZEUTEC Spectra Analyzer™), which was standardized and calibrated using the Kjeldahl method. Based on stability analysis (AMMI and GGE biplots), D^2 analysis and Principal Component Analysis (PCA), a total of six parents were selected with high protein (>10%) in IG74 and Gandhasala; medium (8-10%) in CO52; and low protein (<8%) in CRDhan315, TKM13 and Vellai chithiraikar. The selected lines were subjected to a 6 × 6 full diallel method to study direct and reciprocal combinations during *rabi* 2021. Thus, thirty hybrids and the respective parents were raised in PBS, Coimbatore, using a randomized block design with two replications during *kharif* 2022. The observations on ten agronomic traits were recorded using SES, IRRI, Philippines (2013) for both diversity and diallel studies. In the F_1 generation, grain protein content varied from 6.87 % (CRDhan315 × Vellai chithiraikar) to 11.66% (IG74 × TKM13) with an average of 8.69 %.

Stability analysis was performed based on AMMI (Gauch *et al*., 2008) and GGE biplots (Yan *et al*., 2000) using PBTools version 1.4. The genetic diversity was estimated by the Mahalanobis D^2analysis (Mahalanobis, 1936) using WINDOWSTAT version 7.1 and Principal Component Analysis (Pearson,1901) using GRAPES version 1.1.0 software. The diallel analysis, Method I (F_{1s} + F_{1s} reciprocal and parents) and Model I (fixed effect) of Griffing (1956) and Hayman's (1954) was performed to determine the gene action governing the protein content and other traits. The ANOVA and combining ability were performed using the TNAUSTAT statistical packages.

Results and Discussion

All 169 diverse rice genotypes displayed distinguished variation for grain protein and yield-related traits. Though all three environments had low levels of available soil nitrogen content, the Coimbatore location had relatively higher available soil nitrogen content with

Table 1: Meteorological data from different locations used in the study

Location	Latitude rainfall	Longitude	Altitude	Average	Soil type	N (kg/ha)	P (kg/ ha)	K (kg/ ha)
Coimbatore	11° N	77° E	427 m MSL	670 mm	Clayey	225	20.5	585
Paiyur	12°21′ N	78°18' E	490 m MSL	918 mm	Red loamy sand to sandy loam	112	14.7	329
Aduthurai	11° N	79° E	19.5 m MSL	1139 mm	Alluvial clay	194	20.3	615

medium P and high K than the other two locations (Table 1), which was reflected by higher grain yield and grain protein content as compared to the rest of the locations. As soil fertility status of RRS, Paiyur was relatively low, preferably in terms of available soil N, the yield level and grain protein content were also recorded to be low as compared with Coimbatore and Aduthurai. Nitrogen content is positively correlated with grain yield and grain protein content (Liang *et al*., 2021). The pooled mean of grain protein over three environments ranged from 6.45% in Varigarudan samba to 10.77% in IG74, followed by 10.69% in Gandhasala. The genotypes Gandhasala and IG74 have higher protein content (>10%) and greater single-plant yield, as previously reported by Dhanuja (2021) and Jangala *et al.,* (2022). And hence, these two genotypes could be used as donors to enhance protein content. Concerning the yield-related characters, the number of filled grains per panicle ranged from 38 (IG43) to 159 (Sembarai) and for thousand-grain weight, Mapillai samba recorded the highest (29.08g), and Jeeraga *samba* recorded the lowest (10.08 g).

The results of AMMI and GGE biplots indicated that, out of 169 genotypes, IG74, Gandhasala, Ponkambi samba, Purple puttu, and Burma kavuni performed stably with respect to increased protein content (>10%) across all three locations (Fig. 1). The PCA analysis also showed a similar trend (Fig. 2). Hence, these genotypes can be considered for further breeding purposes for grain protein improvement. The combined D^2 analysis across locations adopting Toucher clustering grouped the genotypes into eleven clusters with a cut-off value of 107.53 (Fig. 3). Most of the landraces were grouped in cluster III, and cluster V might be due to the close genetic relationship among themselves (Ariharasutharsan *et al*., 2022). It was quite interesting to observe that the parents selected based on mean performance were distinctive in such a way that they fell into different clusters *i.e.,* cluster IV (IG74) and cluster XI (Gandhasala) contain genotypes with high protein, and cluster V with low and medium protein (CRDhan315, Vellai chithiraikar; CO52, TKM13) and These genotypes with their unique traits can be utilized for hybridization to exploit transgressive segregants for grain protein content, leveraging their distinct nature to combine different alleles in a single population. The inter-D2 cluster distances were highest between clusters I and XI (D = 56.22), and the maximum intra-cluster distance observed was in cluster X (D = 21.64). Notably, maximum values of cluster mean were observed, including single plant yield (47.94 g) in cluster II, grain protein content (9.96%) in cluster VI, thousand grain weight (25.03 g) in cluster XI and number of filled grains (112.28 nos.) in cluster II. Therefore, it is suggested to cross cluster II with cluster

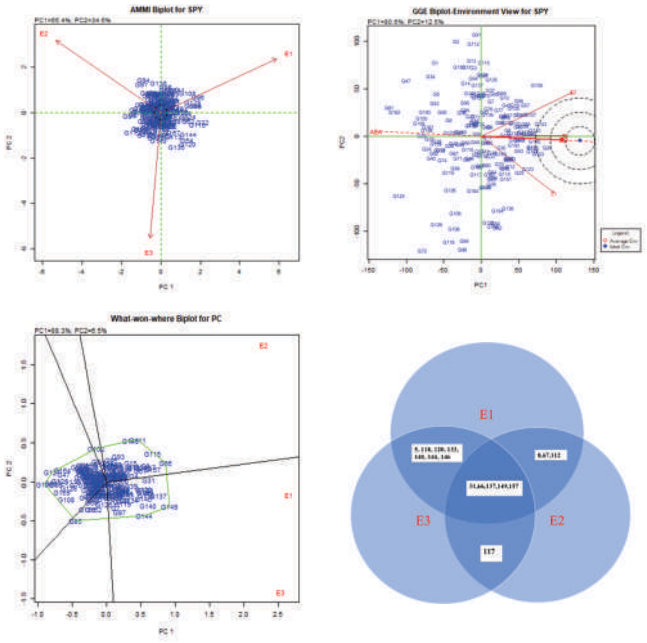

Fig. 1: AMMI and GGE biplots for Single plant yield and Which Won Where Biplot for PC and Representation of Stable genotypes using Venn diagram across three locations. Where, 31 - Purple puttu, 66 - IG74, 137 - Ponkambi samba, 149 - Gandhasala, 157 - Burma kavuni.

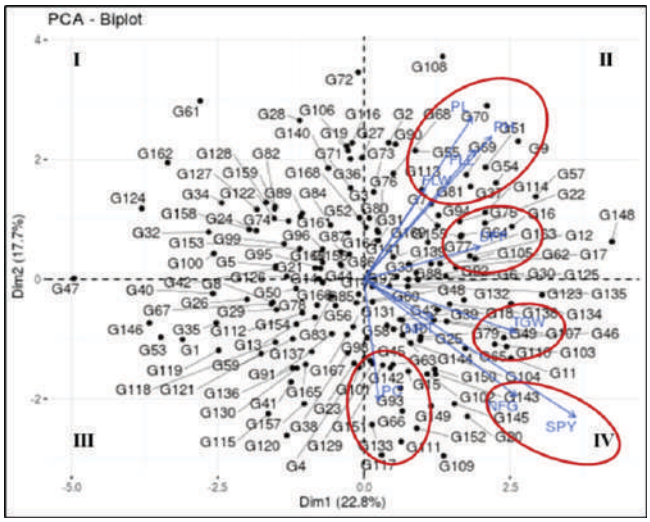

Fig. 2: PCA biplot of 169 rice accessions and 10 traits plotted by PC1 versus PC2 component.

IV to attain superior genotypes with enhanced yield and protein content simultaneously.

Among the studied traits, the number of filled grains per panicle contributed the maximum to the genetic divergence with 71.72%, followed by thousand grain weight (9.59%), whereas the traits panicle length and flag leaf width showed very low contribution to the genetic divergence. The results suggested that for the

selection of genetically diverse accessions, one should categorize the materials based on the traits such as number of filled grains per panicle and thousand-grain weight, as these traits exhibited maximum contribution towards divergence. The 4.28% contribution for PC indicates that protein content contributed less to total divergence but still possesses usable genetic variability. Clusters with higher mean protein content can be exploited through inter-cluster crosses to transfer favourable alleles. Such crosses enhance recombination and indirect selection, increasing the chances of obtaining transgressive segregants with improved protein content.

The same panel with169 genotypes was subjected to PCA analysis and was understood that four Principal Components PC1, PC2, PC3 and PC4 have contributed 68.37% of total variability with eigenvalues of more than one, indicating that these principal components play a major role in creation of variation (Fig. 2). The PC1 component accounting 24.03% of the total variation and has positive loading values for all the traits studied. The genotypes with high PC1 scores are desirable for these traits were identified from the PCA biplot. PCA may not show a clear grouping pattern like D^2 because PCA focuses on variance structure and dimensional reduction, whereas D^2 explicitly measures genetic divergence and enforces clustering. PCA highlights traits with high variance, while D^2 considers cumulative differences across all traits. Traits contributing strongly to divergence may not necessarily explain large variance in the first few principal components. The genotypes Chinthamani, Karuka, Sadai samba, Earapalli samba and Thattan samba on Quadrant 4 exhibited significantly superior values for number of filled grains per panicle, thousand grain weight, single plant yield and IG74. Gandhasala and Vadakathi *samba* exhibited superior grain protein content. Under favourable environments, in genotypes with efficient nutrient use, both yield and protein content may increase simultaneously, resulting in a positive association in PCA. The "yield-protein trade off" is because higher grain yield usually results from increased carbohydrate (starch) accumulation. If nitrogen uptake or remobilization does not increase proportionally, protein concentration becomes diluted, leading to lower grain protein content. Genotypes with favourable yield and grain protein content traits might be utilised in a hybridization program with genotypes from other groups to improve other traits that contribute to overall yield and protein content. Bhargavi *et al*., (2023) have also reported that the rice genotypes with increased protein content, single plant yield and 1000-grain weight may be ideal while making selection for creating genetic diversity. In the present study, the encircling made in biplot exhibited that number of filled grains, single plant yield and thousand grain weight possess close vector angle towards protein content, this might be due to increase in grain density and cell surface area leads to accumulation of more protein content in aleurone layer which is in accordance with the results of Wang *et al*., (2012). While panicle length had a negative association with the number of productive tillers, single plant yield, number of filled grains and protein trait.

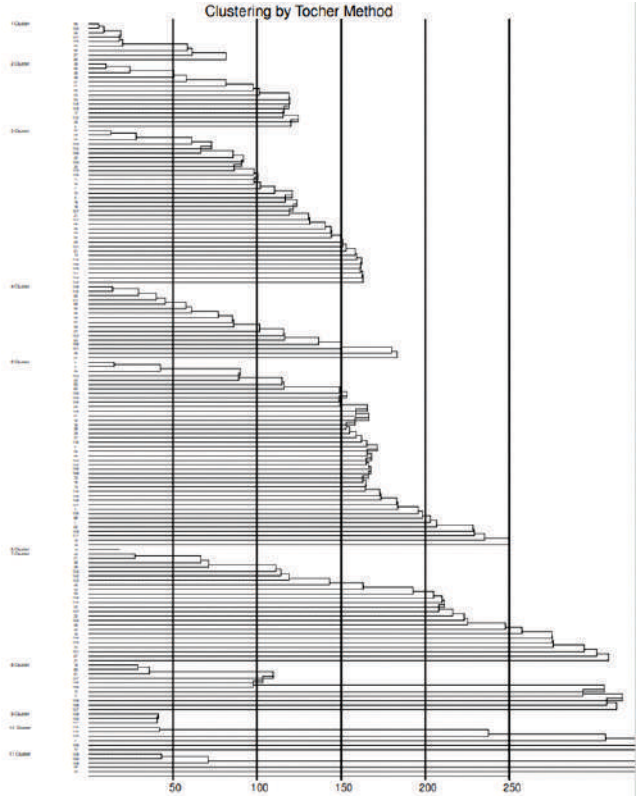

Fig. 3: Clustering of 169 rice genotypes for grain protein and yield related traits by Mahalonobis D^2 analysis over three environments.

The diallel analysis revealed that GCA and SCA variances were highly significant ($P \leqslant 0.01$) for all the traits, including grain protein content. This indicated the role of both additive and non-additive gene effects on the performance of hybrid combinations. In other studies, Bassuony *et al*., (2015) reported that grain protein content was governed by additive gene action and Lingaiah *et al*., (2021) reported that non-additive gene action. The parent IG74 had the highest significant *gca* for protein content, along with thousand grain weight and number of productive tillers with early duration, whereas the parent Gandhasala was adjudged to be the best combiner for protein content along with thousand grain weight. Hence, the good general combiners, IG74 and Gandhasala for grain

Table 2: General combining ability (*gca*) effects of parents for yield and grain protein content

Parents	DFF	PH	NPT	PL	FLL	FLW	NFG	TGW	SPY	TPDB	PC
IG74	-9.56**	-1.74	0.72*	-2.11**	-1.38**	-0.17**	-42.86**	0.33**	-0.07	-13.25**	0.95**
CO52	1.61**	-4.53**	-0.07	0.06	-0.18	0.05	19.60**	-0.08	2.40**	21.45**	-0.46**
TKM13	4.49**	-20.50**	0.10	-2.09**	-2.72**	0.10**	47.26**	-0.85**	-0.25	-12.39**	-0.42**
CRDhan315	1.69**	2.74**	0.51	1.97**	-0.19	0.06	3.51	0.03	-0.66	27.20**	-0.70**
Vellai chithiraikar	-1.18**	23.24**	-1.03**	3.40**	3.09**	-0.11**	-19.40**	1.02**	1.07**	-9.82**	-0.87**
Gandhasala	2.94**	0.78	-0.24	-1.23**	1.39**	0.07*	-8.11**	0.45**	-2.49**	-13.20**	1.50**

* Significant at 5% level, ** Significant at 1% level. DFF-Days to 50% flowering, PH-plant height, NPT-number of productive tillers, PL-panicle length, FLL-flag leaf length, FLW-flag leaf width, NFG-number of filled grains per panicle, TGW-1000 - grain weight, SPY-single plant yield, TPDB-total plant dry biomass, PC-protein content

protein content as well as for important yield-related traits, could be used in the breeding programme to exploit the additive genetic variance (Table 2).

The RCA (Reciprocal combining ability) variance was highly significant for grain protein content and other yield traits, indicating the contribution of the maternal effect on grain protein content, *i.e.* the influence of the maternal parent, to the phenotype of its progeny extends beyond the equal genetic contribution expected from each parent. The endosperm maternal effect for protein content was earlier narrated by (Shi *et al*., 2000; Anyanwu *et al*., 2015) since the grain protein mainly depends on triploid endosperm which derives two genomes from its maternal and one genome from its male parent. This also indicates the probable role of additive gene action for rice grain protein content. Therefore, care should be taken to fix the male and female parents due to the influence of maternal cytoplasm. While examining the mean performance and *rca* effect (Table 3) for grain protein content, the crosses having high *gca* × low *gca* (IG74 × TKM13), (IG74 × CRDhan315) and (Gandhasala × Vellai chithiraikar) were found to have significant mean performance as well as significantly influenced by maternal inheritance for protein content. This indicated that the donors for high protein content should only be used as female parents in the breeding programme to enhance grain protein content to exploit the maternal influence. In direct crosses, having low *gca* × high *gca,* (TKM13× IG74) and (CRDhan315 × Gandhasala), were found to have significant mean performance with a positive significant *sca* effect. These crosses can be

Table 3: Comparison of mean performance and maternal effects in direct and reciprocal cross for grain protein content

Reciprocal cross	Mean	Rca	Direct cross	Mean	SCA
IG74×CO52	10.13*	-0.35*	CO52× IG74	8.51	-0.69**
IG74×TKM13	11.66*	0.92**	TKM13× IG74	9.6*	0.76**
IG74× CRDhan315	9.77*	0.31*	CRDhan315× IG74	9.47*	-0.10
IG74× Vellai chithiraikar	7.63	1.10**	Vellai chithiraikar× IG74	7.7	-0.41*
IG74× Gandhasala	10.95*	0.27	Gandhasala× IG74	9.2	-0.17
CO52×TKM13	7.38	0.08	TKM13× CO52	7.8	-0.33
CO52× CRDhan315	7.48	0.09	CRDhan315× CO52	7.99	-0.76**
CO52× Vellai chithiraikar	7.61	0.70**	Vellai chithiraikar× CO52	7.1	0.07
Gandhasala× CO52	9.31	0.16*	CO52×Gandhasala	7.69	0.19
CRDhan315×TKM13	8.46	0.03	TKM13× CRDhan315	8.4	0.25
Vellaichithiraikar×TKM13	8.05	0.04	TKM13× Vellai chithiraikar	7.32	0.15
Gandhasala×TKM13	8.76	-0.10	TKM13 × Gandhasala	8.57	-0.72**
Vellaichithiraikar× CRDhan315	8.58	0.35*	CRDhan315 × Vellai chithiraikar	6.87	0.49**
Gandhasala×CRDhan315	8.5	0.12	CRDhan315 × Gandhasala	10.47*	0.48**
Gandhasala× Vellai chithiraikar	10.14*	0.61**	Vellai chithiraikar × Gandhasala	7.22	-0.10

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

adopted for recombination breeding in which the selection can be done in later generations to exploit both additive and nonadditive genetic effects. Interestingly, none of the high × high *gca* combination recorded a significant positive *sca* effect for protein content, depicting environmental interaction over the parental lines and hybrids. The lack of positive SCA in high × high GCA combinations for protein content is due to the predominance of additive gene action and environmental influence, rather than fixation alone. Such traits are better improved through selection in segregating generations rather than exploiting heterosis.

Considering the overall performance, IG74, Gandhasala, Ponkambi samba, Purple puttu and Burma kavuni were the stable high protein content genotypes across all locations. The genotypes IG74 and Gandhasala were found to be the best parents for improvement of grain protein content coupled with grain yield. Since the grain protein was influenced by additive as well non additive gene action, hybridization followed by postponement of selection in later generations could be rewarded. As the grain protein content was found to be influenced by maternal genes, the high protein-containing genotypes are recommended to be used as female parents in breeding programmes to cope with maternal influence.

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RESEARCH ARTICLE

Ethnobotany of Job's Tears (*Coix lacryma-jobi* L.): A Vanishing Minor Cereal Crop of NEH Region, India

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Abstract

Job's tears (*Coix lacryma-jobi* L.), a potential minor cereal crop, has been cultivated primarily under shifting cultivation (*Jhum*) in the northeastern hill region (NEH) of India for a long time. At the same time, a decline in crop cultivation has been observed over the years for various reasons. Keeping this in view, an ethnobotanical study was carried out to document the traditional knowledge associated with Job's tears. For this purpose, 45 respondents from 26 tribes across 5 states were interviewed during the study. Based on the information obtained, the crop is mainly grown to fulfil the basic requirements of food, fodder, medicine, local beverage and various other purposes. In addition to these, a cultural festival, '*Mimkut*', with reference to its use, is also celebrated among the Kuki tribe. Besides ethnobotanical information, the rapid decline of its diversity due to changes in consumer preferences, land use patterns and adoption of Christianity by the majority of the tribal communities has also been discussed.

Keywords: Conservation, Ethnobotany, Shifting cultivation, Traditional Knowledge, Tribe

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Introduction

Since time immemorial, plant wealth has played an important role in meeting various kinds of daily needs of human beings. The study of plant resources, which are used by "primitive/aboriginal people or tribal communities", is termed 'Ethnobotany' (Mehta *et al*., 2008; Balick & Cox, 2020). It is a multidisciplinary branch of science, which mainly encompasses botany, anthropology, economics and linguistics. These disciplines study how a society relates to its environment, where indigenous knowledge and its practices play significant roles. One of the fundamental ideas of ethnobotany is that many cultures have formed distinctive knowledge about the crops/plants in their particular environment, which has been transferred from one generation to another by oral tradition (Cotton, 1996). Such studies can be used to compare societies pertaining to their folk knowledge and culture, which may further help in creating theories on origin, migration, diffusion, etc. India is home to over 550 tribal communities having rich traditional knowledge associated with various plants, including minor crops, whose potential has not yet been completely realized as they are produced customarily for a variety of purposes (Bhatt *et al*., 2009). With changes in climatic conditions, risk of pests and diseases will definitely be increased, and if an increase in demand for food crop

production is experienced due to global climate change, then certainly locally grown little known crops like Job's tears (*Coix lacryma-jobi* L.) will function as a staple crop to ensure food security, healthcare and economic upliftment of rural/ farming /tribal communities.

C. lacryma-jobi (2n = 4X = 20) belongs to the family Poaceae, is commonly known as bodhi pearl, millet, pearl rice, medicinal corn, *Coix* pearl, etc. It is also called *Adlay* in Filipino, *Sankru* in Hindi, *Jargadi* in Sanskrit, and *Gurgur* in Bengali (Hore and Rathi, 2007). It is an annual, photosensitive grass that thrives in cool, damp, marshy land and acidic soils with moderate temperatures, and can also withstand high rainfall conditions. Its flowers are monoecious, seeds/ grains are hard, with a shiny coat, and have a hole at the tip from where the flower emerges. The whole plant and its seed pod are commonly known as Job's tears because the seed pod often has a teardrop appearance. Job's tears wild form (hard and stony, fruit case strong, unbreakable, polished/shining, perennial forms), var. *stenocarpa* (plants akin to cultivated forms with coarse leaves, fruit hard and stony, elongate-cylindrical), var. *monilifer* (a variation of *stenocarpa* with more variable fruits); and cultivated form (thin shelled, fruit case soft, breakable, coarse, not shining, bold) and var. *ma-yuen* (tall, annual, all soft-shelled types, fruit case pear shaped to spheroidal) occur in a similar phytogeographical range with various intergrading types. The crop was grown as a minor cereal in the remote part of the northeastern hill region (Arora, 1974), which is also considered the centre of origin/diversity of this crop (Ahmed and Singh, 2007). Besides the NEH region, it is also cultivated in China, the Philippines, Burma, Sri Lanka, and some other Asian countries. It is a multipurpose crop, which is nutritionally superior to most cereals and millets in terms of protein and other vital minerals like calcium, zinc and iron (Venkateswarlu and Chaganti, 1973). Documenting ethnobotany and comprehen-ding the traditional knowledge associated with the crop may aid in the conservation of its genetic resources by encouraging the sustainable land management techniques that can uphold the rights and autonomy of indigenous/tribal people (Thomas *et al*., 2011; Pradheep *et al*., 2014).

Materials and Methods

For recording ethnobotanical information/ associated traditional knowledge, and factors responsible for its genetic erosion, surveys were conducted in five states of NEH region (Table 1). To record the baseline information, structured and unstructured questionnaires included a set of questions regarding the basic details of the farmer, farming system adopted, part used, vernacular names, local uses, method of usage and recipes prepared, as well as the causes of genetic erosion of the crop were used (Pareek *et al*., 2000). During the survey, a total of 45 respondents (23 male and 22 female) between age groups of 20-80 years, belonging to 26 tribes, were interviewed. Prior informed consent was taken from the *Gram-bhuda* (*Sarpanch*) before collecting the data from households. The qualitative data obtained were analyzed using Microsoft Excel for descriptive statistics, while the findings were summarized using charts and graphs. The recorded information was compared with the reported information for authentication and new uses. Herbarium specimens of distinct and wild material were identified with taking help of expert taxonomists and deposited in the National Herbarium of Cultivated Plants (NHCP) located at ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi.

Table 1: Details of states and districts surveyed and number of respondents interviewed

State	Districts surveyed	Tribes interviewed	No. of respondents
Arunachal Pradesh	West Siang, East Kameng, Siang, East Siang, Dibang Valley	Galo, Nyishi, Adi (Bori), Idu Mishmi	10
Manipur	Churachandpur, Bishnupur, Imphal West, Kamjong, Senapati	Kuki, Meitei, Poumai-Naga, Tangkhul, Paite	10
Meghalaya	South Garo hills, West Garo hills, East Khasi Hills, West Jaintia hills, Ri-Bhoi	Garo, Khasi, Jaintia, Bhoi	8
Mizoram	Aizawl, Mamit	Mizo, Mizo Lushai	5
Nagaland	Mokokchung, Noklak, Kohima, Peren, Phek, Dimapur, Peren, Zunheboto, Phek	Rengma, Ao, Khiamniungan, Chakhesang Angami, Zeliang, Pochury, Kachari, Kuki, Sumi, Chakhesang	12

Results and Discussion

In NEH region, Job's tears is majorly growing under shifting cultivation as informed by the respondents (76%), followed by sedentary farming (15%) and kitchen garden (9%) (Fig. 1). Shifting cultivation (also known as 'jhum') (Fig. 2 a,b) is an important indigenous form of cropping system practiced by the farming communities in the hills of NEH region, which is a traditional method of cultivation being followed since long past (Bhatt *et al.*, 2024). Owing to the mountainous terrain, settled cultivation is mainly limited to plain areas, which constitute a small portion of the cultivable area. However, in the NEH region, the kitchen garden has cultural significance, hence plays a vital role in maintaining crop biodiversity (Tripathi and Barik, 2003), where women are contributing the most in the cultivation of crops to fulfil the various needs and earn money through the sale of their produce in local markets (Shimrah *et al*., 2018). *C. lacryma-jobi* is known by different vernacular names by tribes of different states *viz.,* in Arunachal Pradesh : *Adi - Tanyet; Adi (Bori) - Tatsing; Ibu Mishmi - Neh*; in Manipur : *Kuki - Mimchang; Paite - Ngabem; Poumai Naga - Duveio; Tangkhul - Atheitha; Thadou - Mimchang*; in Meghalaya : *Khasi-Sohriew; Garo - Megaru; Jaintia - Garada; in Mizoram : Mizo - Pingpih; Mizo Lushai - Mim*; in Nagaland : *Angami - Keshi; Ao - Menchong; Chakhesang - Kiise; Kachari - Lukuthui; Khiamniungan - Linyak; Kuki - Mim; Pochury - Asheta; Rengma - Nsha*; and *Sumi - Akithi*. These names are the manifestations of man's long-standing association with his surrounding green environment (Bareh, 2001). Local names given to plants by indigenous people in their local dialects often reflect a broad spectrum of information on their understanding. Most often, the local names are given based on some salient features, e.g., appearance, shape, size, habit, habitat, smell, taste, colour, utility, and other peculiar characters, etc. of the plants (Bhatt *et al*., 2022). In earlier days, almost every household of the NEH region used to grow this crop, but presently it is vanishing from the entire jhum areas. A significant amount of genetic diversity from the farmer's field can be lost in future, if efforts are not made for its timely conservation (*ex-situ/in-situ* on-farm) and use in breeding programme (Laxmisha *et al.,* 2022; Soni *et al.,* 2023). Hence, documentation of traditional knowledge associated with the crop is a need of the hour.

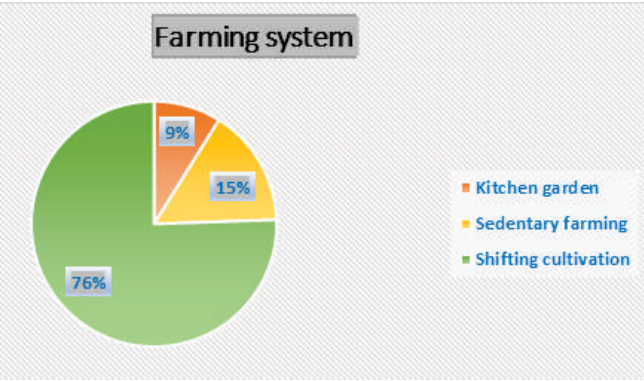

Fig. 1: Farming system followed for Job's tears cultivation.

Fig. 2 (a): Job's tears cultivation in 'jhum field'. **Fig. 2(b):** Seed variability in Job's Tears.

Local/ethnobotanical uses of Job's tears

Generally, plants from the surrounding green environment have been used to fulfil various needs by human beings. Similarly, Job's tears is also used in many ways by the tribes of NEH region (Fig. 3). During survey, majority of the respondents (36) informed that its grains are used as food and ornament (15), leaves as fodder (12), medicine (4), feed (4), fertilizer (3) and dried plant as fuel (1). Many tribes are also using its different parts for medicinal purposes (8), and as narrated by the locals, the crop has religious/cultural significance (4) too. Below are some examples of the local uses of the crop as recorded from the experienced local folks of the NEH region.

Food: Thirty-six respondents (Fig. 3) informed about the use of grains in the preparation of various food items and local beverages. The matured grains are cooked with rice; dehulled grains with other cereals are

Fig. 3: Local uses of Job's tears as narrated by respondents.

boiled in water and cooked as porridge and *khichree* (Fig. 4a); soaked grains are roasted and popped like popcorn; grains are soaked in water for a whole night, sun dried and pounded to make dough and bread/chapatee, whereas sometimes kneaded flour is used to make dough, which is boiled and eaten; the flour of grains is mixed with water and consumed as a refreshing drink (Hore and Rathi, 2007); whole grains are soaked into water for overnight, dried in sunlight and fried in oil to consume as snacks; grains are boiled in water and added with sugar/ salt to make tea. During scarcity, the Sulung tribe of Arunachal Pradesh used to gather seeds of wild form and cook along with grains of *Echinochloa colonum* (Barnyard millet) and *Panicum milliaceum* (Proso millet) to make pancakes (Singh, 2008).

Beverage: The tribes of the NEH region consume unique food items and also follow their own methods of preparing beer or traditional beverages from food items/grains (Bareh, 2001). Unlike cereals and millets, Job's tears is also used in the preparation of a local beverage. The traditional beverages are meant not only for consumption but also for using as medicine and bestowing it to their deities (Gangwar & Ramakrishnan, 1990). Consumption of fermented beverages and alcoholic drinks has a deep-rooted history among different cultures. These drinks are widespread because of their nutritive value as well as the pleasure they impart, which makes them the most acceptable products for consumption during various religious or recreational rituals (Gogoi, 2021). The method of traditional alcohol brewing is different among the tribes as they follow their own unique protocol using distinct starter cultures, even though the majority of them use a similar base for fermentation (Tamang *et al*., 2016). The *Zeliang* tribe (Nagaland) use grinded grains for preparing local beer '*Hechi*' (Fig. 4b). For the purpose, local rice grains mixture is kept inside the dried seedless fruit of bottle gourd (locally known as *Kela*) for a period of four days or up to one month (Fig. 5a) along with starter culture (locally known as 'medicine'). This beer can be kept for consumption till one week. The *Ao* tribe (Nagaland) prepares two grades (superior and inferior) of alcoholic drink. In superior grade, grains are boiled and 'medicine' (starter-which is a mixture of rice and fungal culture) (Fig. 5b) is added and kept for three days to initiate the fermentation process, while consumed when the froth comes out. Whereas in the inferior grade, the leftover mixture of grains from the superior one is crushed into a paste and water is added to make a drink before consumption. In the *Angami* tribe

Fig. 4(a): Job's tears dehulled grains.

Fig. 4(b): Job's Tears beer '*Hechi*' of *Zeliang* tribe.

Fig. 5(a): Bottle guard in preparation of Job's Tears beer.

Fig. 5(b): Local recipes of Job's Tears.

(Nagaland), a wine '*Kesikhe*' is prepared, for which the mixture of grains is added with starter culture and kept for two months for fermentation. This wine is special and shared only among loved ones, but at present its preparation is declined, hence many people have not tasted it to date. The Sumi tribe of Nagaland brews a wine '*Akithiji*' using its three month's old fermented seeds. Similarly, wine '*Shethaka*' prepared by the Pochury tribe of Nagaland, beer '*Nshazu*' by the Rengma tribe of Nagaland and wine '*Noyin*' by the Adi Bori tribe of Arunachal Pradesh are prepared by fermenting dehulled grains of Job's tears. It was observed that adoption of Christianity by most of the tribal communities in Nagaland during the late 20th century, the consumption of locally brewed alcohol was forbidden because the consumption of alcohol was considered equal to sin. Hence, many tribal communities (Pochury, Zeliang and Sumi) of Nagaland have abandoned the cultivation of this alcoholic beverage brewing crop (Dhanaraju, 2019).

Medicine: Besides food and beverages, it is also used in the local medicine system (Fig. 3). Still majority of tribes rely on the community-based traditional healing system for their basic healthcare needs in the NEH region (Ghosh, 1992). The Poumai-Naga tribe of Manipur use its root paste with honey and milk in the treatment of fever and as a vermifuge. The root infusion is used by the Meetei community of Manipur as a blood purifier (Singh & Pilgrim, 2010). The leaf juice is used to treat diarrhoea and dysentery by the Garo tribe of Meghalaya, while the paste of fermented seeds is applied externally to treat skin diseases and chickenpox. The decoction made from both leaves and roots is used in the treatment of urinary tract infection by rural communities of Mizoram (Laxmisha *et al*., 2022). The seeds/grains are boiled with milk or water and are given by the Angami tribe of Nagaland in the treatment of kidney problems and asthma. The boiled grains are also given to people who are suffering from throat cancer. Freshly plucked leaves are boiled in water and drunk as green tea, which is supposed to be good for indigestion. The Pochury and Kachari tribes of Nagaland use cooked grains as rice and give it to the people with high blood pressure, while the decoction of leaves is orally taken for treating kidney problems. The Chakhesang tribe of Nagaland consume its grain to reduce joint pain; decoction of fresh leaves is also taken as a blood purifier; and leaves are dipped into hot water and later taken in a cloth and put on pain areas in joint pain. In the Garo tribe of Bangladesh, *Coix gigantea* root paste is used for the treatment of leprosy (Ningombam *et al.*,2014).

Ethnic ornaments: *Coix* gives the most perfect beads for various ornamental purposes. The seeds obtained from wild types have been used by rural folks to make different kinds of ornamentals like earrings, rosaries, garlands, rings, bracelets, belts, photo frames, etc. Its seeds are an excellent source of natural jewellery as it has a hole in the centre through which wire or thread can easily pass. The traditional headgear of the Mizo women, called '*Vakiria*', is decorated with *Coix* seeds during celebrations (Laxmisha *et al*., 2022). Fifteen respondents from different tribes like Nyishi of Arunachal Pradesh; Paite tribe of Manipur; Jaintia of Meghalaya and *Mizo* of Mizoram have informed that chains, necklaces and bracelets are made from wild type of hard seeded *Coix* using strings; whereas in many places, its seeds are also used to decorate the belts (Fig. 6 a,b). The seeds can also be used in making musical instruments such as Shaker's gourd by covering hollow gourds with a loose net strung with beads of *Coix* (Anisuzzaman *et al*., 2007).

Fig. 6(a): Ornaments from the wild type of Job's tears.
Fig. 6(b): Balls from grains used as feed of pigs.

Fertilizer/manure and fuel: Different tribes like Adi of Arunachal Pradesh, Khasi of Meghalaya and Kuki of Nagaland use decomposed leaves and stems as manure. The quality of manure is improved when grains are trampled in piggeries and mixed with mud (Biswas & Das, 2022). Dried leaves and stems are burnt as fuel by the Kuki tribe (Nagaland) and also stored for off-time.

Animal feed and fodder: Dehulled/polished grains are used to feed pigs and hens by the Thangkul tribe of Manipur and the Chakesang tribe of Nagaland. Presently, the soft-shelled types of Job's tears are gaining popularity as feed for poultry and rabbits (Arora, 1977). The Bhoi tribe of Meghalaya, the Galo of Arunachal Pradesh and the Mizo Lushai of Mizoram use green leaves and stems of the plant as fodder for cattle and pigs. The green leaves can be used to make silage (Gupta *et al*., 1995).

Religious/cultural value: Apart from food, medicinal and other ethnobotanical uses, the crop holds cultural

and religious significance among many tribes of NEH. Its tear-shaped seeds are primarily used as beads for making prayer beads, rosaries, and garlands. The seeds of wild species are called *Vaijayanti*, which are considered sacred because a garland made from their seeds is worn by the gods Vishnu and Krishna. In Manipur, garlands made from seeds of wild (*Coix stenocarpa)* are used for religious purposes by the Meitei tribe. Similarly, the Muslim community of Manipur use a wild form of Job's tears locally known as '*Tasbih Pambi*' for counting the number of prayers daily by righteous men (*Tasbih Chatpa*) (Jain & Banerjee, 1974). During the 'Hega festival' of the *Zelaing* tribe of Nagaland, its beer is commonly served among the people. Among the Kuki tribe of Nagaland, the main festival '*Mimkut*' ('*Mim*' referred to as Job's tears, while '*kut*' as the post-harvest) is celebrated immediately after the harvest of Job's tears. In NEH, the rich cultural history associated with it is still preserved in the memory of tribal communities and expressed through folktales, folksongs, regional paintings, and other artefacts (Ahmed & Singh, 2007). 'A story of two brothers', associated with Job's tears, is exemplified by the people in each generation among the Kuki tribe of Nagaland. As per the story, Mr Lendou and his younger brother, Mr Teucha, who were abandoned by their mother, share a piece of *Mim* to fulfil their feeling of hunger that expressing brotherhood and love with each other in scarcity of food as well as in a state of distress. The Pochury tribe of Nagaland gather around the jhum field and sings harvesting songs as the crop is harvested.

Other uses: Grains are used to trap the rats when they attack the crop, while the stems are used for thatching the huts by the *Khasi* tribe of Meghalaya. The children of the *Kachari* tribe of Nagaland use grains of wild form known as '*Kawrimoni*' for counting when playing a game. Grains are also used as bait in '*Riam Kallong*', a traditional method of fishing among the *War Khasi* community of Meghalaya (Bhalerao *et al*., 2016).

Though majority of information is available in the literature but some of the ethnobotanical information recorded first time from the tribal communities of NEH region are popping grains for consumption; method of preparation of local beverages from its grains like *Hechi, Keshike, Noyin, Shethaka, Akithiji,* etc.; use of fermented seed pastes in treating chickenpox, usage of leaf paste in joint pain; use of grains in local festivals Hega festival, Mimkut festival; using grins of wild form locally known as *Kawrimoni* for counting; folktale regarding two brothers and harvesting song of Job's tears are.

Conclusion

Ethnobotanical study of Job's tears indicated that the crop has significant importance in the culture and day-to-day life of the people of the NEH region. Job's tears seeds are a rich source of nutrients and justify the food status. Hence, it has been used by the tribes as food, fodder, medicine and ethnic ornaments, etc. Discontinuation of crop cultivation is a serious concern; hence, both *ex situ* and *in situ* on-farm conservation efforts need to be expedited to enhance the sustainable management of such valuable /potential crop. As the crop is highly nutritious, the development of value-added products will certainly help in doubling the farmer's income. Therefore, fine-grid surveys need to be undertaken in remote parts of the NEH region for the collection of its untapped germplasm before it is lost forever. Validation of the ethno-medicinal use of Job's tears for the development of new medicines is the need of the hour. Further studies need to be undertaken to evaluate and determine the use of this crop in aspects of medicinal, nutraceutical and pharmacological values. Hence, efforts for conservation (*ex-situ, in-situ*, on farm) and use for developing superior genotypes are essential.

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RESEARCH ARTICLE

UTR-181: A Fine Grain, Long Duration, Blast Resistant Rice (*Oryza sativa* L.) Culture Suitable for Irrigated Ecology of Andhra Pradesh

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Abstract

UTR-181 is a fine-grain rice culture more suitable for irrigated ecology, and being a long-duration culture is furthermore appropriate for Single Cropped Areas of the Andhra Pradesh state. It is derived from a cross of NLR-34449/MTU-1010 through the Pedigree method of breeding at Agricultural Research Station, Utukur, Kadapa. It recorded 10.1% yield improvement over the check BPT-5204 and exhibited 21.2% improvement over RGL 2537 in Station, Multilocation trials, minikit testing across the state of Andhra Pradesh. It has a non-lodging nature, high yielding, nitrogen responsive, with medium green foliage, low shattering and exhibits complete exertion of panicle. It is also tolerant to leaf blast, neck blast in comparison with the popular check BPT-5204. This variety has excellent cooking quality, intermediate amylose content and glycemic index (65.6). It also possesses essential grain characters like high head rice recovery (more than 65%) with translucent grains to get a premium market price.

Keywords: Rice, Variety, Yield

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Introduction

Rice (*Orya sativa* L.) is the staple food, and it can be adapted to diverse agro-climatic conditions throughout the world. In India, rice is cultivated in an area of about 44 million ha with a total production of 129 million tonnes and a productivity of 6214 kg/ha. In Andhra Pradesh, it is cultivated in 25 lakh hectares in the *kharif* and *rabi* seasons. The KC Canal belt is one of the most important rice growing zones of Andhra Pradesh, where rice crop has been cultivated for three seasons viz., early *kharif* (April-August), late *kharif* (August-January) and *rabi* (November-March), depending on the availability of irrigation water for rice cultivation. In the *kharif* season, short-duration (120-125 days), in late *kharif*, long-duration (150 days) and in rabi, medium-duration (130-135 days) varieties were generally cultivated in this area. BPT-5204 is predominantly cultivated in the Kadapa district of Andhra Pradesh.

Though BPT-5204 possess good yield potential and excellent grain & cooking quality, it is susceptible to major pests and diseases, due to which farmers often suffer heavy yield losses. Hence, there is a demand from the farming community

for stable rice varieties with sustained production even under adverse climatic conditions, with reduced cost of cultivation. Efforts were made at the Agricultural Research Station, Utukur, for the development of high-yielding, non-lodging, low-shattering rice varieties along with resistance to leaf blast, neck blast, plant hoppers, coupled with good grain quality. Many varieties possessing high yield potential with tolerance to blast and BPH were released for the replacement of Samba Mahsuri (BPT-5204). But, many of them were not accepted by farmers due to their poor cooking quality traits. UTR-181 possess excellent grain and cooking quality parameters similar to Samba Mahsuri.

Materials and methods

UTR-181 rice culture was developed at ARS, Utukur, Kadapa, ANGRAU, by following the pedigree method of breeding. This culture is a derivative of NLR-34449 × MTU-1010. This is a long-duration culture, and the growing season is July to August. It was tested for yield and its attributes at the station level yield trials from 2015-16 to 2017-18. The culture was tested in Multilocation testing in ANGRAU during the *kharif* season of 2018-19 and 2019-20 under slender grain trial. UTR- 181 (IET 30082) was tested in AICRIP testing during *kharif* -2021 across 19 locations in the country. It was tested for pests and diseases in the AICRIP under NSN 2 nursery. It was tested in farmers' fields under minikit testing from 2020-21 to 2022-23 for three years in 42, 220, 255 locations throughout the state in comparison with the various checks, such as BPT 5204 and RGL 2537. The data on fifteen quality parameters in comparison with the check BPT 5204, as conducted at ARS, Nellore, during 2021-22. It was deposited as an indigenous rice culture and got IC number (IC 650223) for further reference. The DNA fingerprinting data were generated by using different markers at RARS, Maruteru, and ANGRAU.

Results and discussion

The hybridization between NLR-34449 × MTU-1010 was attempted during 2009. Superior progeny was identified in F_6 generation and yield trials were conducted at station level for 3 consecutive years from 2015-16 to 2017-18 and it recorded an average grain yield of 8898 kg/ha as against the checks NLR-34449 (6034 kg/ha) and BPT-5204 (6560 kg/ha) which is 48.50% and 33.70% increase over the checks, respectively. It was tested in multilocation testing under the Slender Grain segment during *kharif* 2018 and late segment during *kharif* 2019 at the station level, and it recorded an average grain yield of 7198 kg/ha and 6415 kg/ha, which is a 7.37% and 13.10% increase over the check BPT-5204 (6704 kg/ha and 5670 kg/ha, respectively). Multilocation testing was also done in 8 centres against the check BPT-5204, wherein it recorded an average grain yield of 5908 kg/ha, which is a 16.2% increase over the check BPT-5204 (5084 kg/ha) variety used. In the year 2019, again it was tested in MLT in 9 centres against BPT-5204 and MTU-1190 checks, wherein it recorded 5711 kg/ha, which is 19.2% and 7.2% superior to the checks (4788 kg/ha & 5327 kg/ha, respectively) used. The overall mean of the culture was 7418 kg/ha (Table 1).

The performance of any culture was proven when it was tested on a large-scale area in the farmers' field. The culture was tested through DAATT centres and the Department of Agriculture throughout Andhra Pradesh

Table 1: Performance of UTR-181 in station yield trials and multi-location trials at ARS, Utukur

Name of the trial	Code testing	Year of testing	Grain yield (kg/ha)		Per cent increase over check	CD (0.05)	CV (%)	
			UTR-181	Check				
OYT (ARS, Utukur)	UTR-181	*Kharif* 2015	8750	5833 (NLR34449)	50.0	2489	16.6	
PYT(ARS, Utukur)	UTR-181	*Kharif* 2016	9176	6234 (NLR34449)	47.2	1024	7.01	
AYT (ARS, Utukur)	UTR-181	*Kharif* 2017	8769	6560 (BPT-5204)	33.7	700	5.35	
MLT-Slender grain (ARS, Utukur)	Sg386	*Kharif* 2018	7198	6704 (BPT-5204)	7.37	530	4.73	
MLT-Late (ARS, Utukur)	Sg412	*Kharif* 2019	6415	5670 (BPT-5204)	13.1	1116	7.40	
MLT-SG (I year) (8 locations)	Sg386	*Kharif* 2018	5908	5084 (BPT-5204)	16.2	-	-	
MLT-SG (II year) (9 locations)	Sg412	*Kharif* 2019	5711	4788 (BPT-5204)	19.2			
				5327 (MTU 1190)	7.2	-	-	
Mean			7418	5839	27.0			

for three consecutive years from 2019-20, 2020-21 and 2021-2022 under minikit testing in 42, 220 and 255 farmers' fields, respectively. In minikit trials, the culture was tested against respective rice varieties grown in that particular area in different districts of Andhra Pradesh, wherein it recorded an average grain yield of 6209 kg/ha as against the check 5601 kg/ha, which is a 10.9% increase over the check (Table 2).

During *kharif* 2021, UTR-181 was nominated and tested as IET 30082 along with 63 entries under IVT-L trial in 19 centres all over India under AICRIP testing, along with two checks, BPT-5204 and ADT-49. It recorded an average grain yield of 4784 kg/ha with the highest yield of 12153 kg/ha at Kurumbapet centre. UTR-181 recorded an increased grain yield of 4.20% over BPT-5204 and 3.70 % over ADT-49 in different centres of AICRIP (Table 3) (IIRR Annual Progress Report on crop improvement 2013, Vol. I, Page Nos. 1.215-1.226).

Disease and pest reaction

The culture was tested for various pests and diseases at the Agricultural research station, Utukur, from 2020-21 to 2022-2023, and it showed moderate resistance to leaf blast (3-4) (Table 4a). It also exhibited a lower percentage incidence and field tolerance to leaf folder (1) and stem borer (1), and BPH (1) in comparison with the popular check BPT-5204 (Table 4b). In AICRIP testing during 2021, it was tested in NSN II nursery, where it was found moderately resistant to neck blast and leaf blast (Table 5a) and exhibited moderate resistance to BPH at Pant Nagar and Gangavati locations. It also recorded

Table 2: Three years of compiled data of UTR-181

Year of testing	No. of Minikits given	No. of Minikits data obtained	Minikit yield (kg/ha)	Check yield (kg/ha)	Per cent yield advantage
First year (*kharif*, 2020)	42	36	6735	6106	10.3
Second year (*kharif*, 2021)	220	213	5708	5177	10.2
Third year (*kharif*, 2022)	255	253	6185	5520	12.0
Total	517	455	6209	5601	10.9

Table 3: Performance of UTR-181 in Initial Varietal Trial- Medium slender under AICRIP testing during *kharif*, 2021

Year of testing	Name of the trial	Location	State	Grain yield (kg/ha)		
				UTR-181	BPT5204	ADT 49
2021-22	Initial Variety Trial-Medium slender	Arundhutinagar	Tripura	5040	4550	4750
		Ranchi	Jharkhand	4255	4721	3657
		Sindewahi	Maharashtra 1	4510	2727	4510
		Karjat	Maharashtra 2	5442	4932	4252
		Sakoli	Maharashtra 1	2845	3507	3474
		Navagam	GUJARAT	2877	2606	3576
		Jeypore	Odisha	2365	2145	1568
		Raipur	Chhattisgarh	2715	3312	4758
		Maruteru	Andhra Pradesh	4735	9410	6745
		Bapatla	Andhra Pradesh	5352	4831	4181
		Rajendranagar	Telangana	2555	3465	4844
		Kampasagar	Telangana	5582	5877	6025
		Aduthurai	Tamil Nadu	7118	5694	6007
		Coimbatore	Tamil Nadu	6736	4880	5256
		Mandya	Tamil Nadu	5244	5567	4656
		Mugad	Karnataka	4342	4165	5869
		Gangavathi	Karnataka	3936	5228	3936
		Sirsi	Karnataka	3100	2500	3367
		Kurumbapet	Puduchery	12153	7143	6250
Average performance of UTR-181 and check varieties over different locations				4784	4593	4615
% increase over BPT5204				4.2		
% increase over ADT49				3.7		

Table 4a: Reaction of UTR-181 to major diseases at ARS, Utukur from 2020-21 to 2022-23

Variety	Average leaf blast score (Range of scores)
UTR-181	3.6 (3 to 4)
Check (BPT-5204)	5.6 (4 to 7)

Table 4b: Reaction of UTR-181 to major insect pests at ARS, Utukur during 2020-21 to 2022-23

Variety	Dead hearts (%) (Score)	Leaf folder (%) (Score)	BPH per hill (%) (Score)
UTR-181	7.83 (1)	9.2 (1)	3.10 (1)
Check (BPT-5204)	14.9 (3)	18.1 (3)	23.4 (3)

Table 5a: Reaction of UTR-181 to major diseases

Variety	National Screening Nursery – II (AICRIP) (testing during 2021)			Variety	At ARS, Nellore
	Leaf Blast (19 locations)	Neck Blast (5 locations)	Sheath Blight (19 locations)		Leaf Blast score
UTR-181 (IET 30082)	6.3	4.8	5.1	UTR-181 (IET 30082)	5.0
Check (BPT-5204)	5.4	4.3	6.1	Check (BPT-5204)	8.0
Resistant check (Tetep)	3.2	1.5	6.0	Resistant check (Tetep)	2.0
Susceptible check (Pusa-44)	5.6	5.5	6.2	Susceptible check (Pusa-44)	6.0

Table 5b: Reaction of UTR-181 to major insect pests

Variety	National Screening Nursery – II (AICRIP) (testing during 2019)	
	Leaf Folder damage (4 locations)	Brown Plant Hopper damage score (2 locations)
UTR-181 (IET 30082)	11.1	5
cCheck (BPT-5204)	11.9	7
National check (WGL-14)	11.6	3
Susceptible check (TN-1)	12.5	7

Table 6: Interaction of grain yield between time of planting and nitrogen levels during *kharif* 2022

	N_1 : 40 kg/ha	N_2 : 80 kg/ha	N_3 : 120 kg/ha	N_4 : 160 kg/ha	Mean
July II F. N	4963	5327	5463	5497	5313
August I F. N	5486	5672	6234	6346	5935
August II F. N	5238	5579	6148	6016	5745
Mean	5229	5526	5948	5953	
		$S.Em_+$	179	224	
		CD (*P*=0.05)	NS	NS	

less incidence of leaf folder in the locations namely Kaul, Karjat and Navsari (Table 5b).

Agronomic evaluation

The culture UTR-181 was tested for four different nitrogen levels and different times of planting during kharif 2022, wherein it recorded 6148 kg/ha at 120 kg nitrogen application per hectare at August II F.N. It responds even up to 160 kg N with planting time of August I F.N., but the optimum dosage is 80 kg/ha (Table 6).

Morphological features

The morphological features of the cultures are given in Table 7. The culture attains 50% flowering at 105-115 days after sowing, and it grows up to a height of 100-110 cm and bearing 18-23 tillers per plant. The panicle length is 25 cm, and the grains were medium slender

Table 7: Description of UTR-181

Trait/character	Description	Trait/character	Description
Plant height	100-110 cm	Lemma palea colour	Straw
Habit	Erect	Lemma palea pubescence	-
Days to 50% flowering	105-115 days	Seed coat colour (bran)	Straw
Lodging	Non lodging	Sterile lemma colour	Straw
Leaf blade colour	Green	Grain type	Medium slender
Basal leaf sheath colour	Green	Unpolished Kernel length (mm)	5.72
Leaf angle	Erect	Unpolished Kernal Breadth (mm)	1.78
Flag leaf angle	Erect	L/B ratio	3.21
Leaf length	45 cm (medium)	1000 grain weight (g)	14.5
Leaf width	1.2 cm (medium)	Gelatinization temperature	Intermediate
Leaf blade pubescence	Medium	Kernel elongation ratio	1.31
Ligule colour	White	Keeping quality	Good
Ligule shape	Split	Grain shattering	Low
Ligule length	3.6 mm	Flowering duration (days)	4-5 days
Auricle colour	White	Dormancy (weeks)	Two weeks
Collar colour	White	Total grains/panicle	400-450
Culm angle	Erect	Tillering ability	High tillering (18-23)
Flag leaf angle	Erect	Distinguishing morphological characters	High-yielding, non-lodging, fertiliser responsive, semi-dwarf with green foliage, grains are light straw glumed, medium slender grain, two-week seed dormancy, low shattering with high grain number per panicle
Culm internode colour	Green		
Panicle length	25 cm		
Panicle type	Compact		
Panicle exsertion	Mostly exerted		
Awns	Absent		
Apiculus colour	Straw		
Stigma colour	White		

Table 8: Grain quality data of UTR-181

Trait/Character	UTR 181	Check (BPT 5204)
Grain type	MS	MS
Kernel length (mm)	5.72	5.13
Kernel breadth (mm)	1.78	1.81
L/B ratio	3.21	2.83
1000 grain weight (g)	14.5	14
Hulling per cent (%)	82.5	78.8
Milling per cent (%)	73.5	70.7
Head Rice recovery	65-70%	57.2
Volume expansion ratio	3.1	3.3
Gelatinization temperature	Intermediate	Intermediate
Water uptake (ml)	250 ml	330 ml
Kernel length after cooking (mm)	8	9.2
Keeping quality	Good	Good
Cooking quality	Very good	Excellent
Elongation ratio (mm)	1.31	1.74
Alkali spreading value	7.0	4.0
Amylose content (%)	24.0	23.4
Gel consistency (mm)	65	24

type with straw colour, and awns were absent. The culture is non-lodging, tall with erect plant type, dark green foliage, fertiliser responsive, low shattering, two weeks dormancy and higher grain yield. Each panicle was fully exerted from the boot leaf and comprises 400-450 grains per panicle.

Quality features

After grain yield, the second most important thing that needs to be considered is quality, which includes physical, milling, cooking and chemical quality parameters. The culture UTR-181 is a medium slender culture with the kernel length of 5.72 mm, breadth 1.78, whereas the kernel L/B ratio was 3.21. The head rice recovery of the culture is >65% and it is an acceptable recovery draw the millers 'point of view. Absence of grain chalkiness and good kernel elongation ratio of 1.31 and volume expansion of 3.1 show a good sign for the cooking quality of rice. The grain size belongs to the medium slender group, and the amylose content (24%) and gel consistency (36 mm) were also under desirable limits (Table 8). In the organoleptic test conducted with 15 individuals, it was found that the keeping quality was very satisfactory and on par with BPT-5204. Its straw is very palatable to cattle.

In view of the above, it was concluded that the culture UTR-181 possess good yielding ability at the station level and also at farmers' fields, good milling and cooking quality traits along with blast resistance, suitable for sowing from July to August. Further, even under delayed transplanting conditions (aged seedlings), it was found to be suitable for cultivation in the irrigated rice ecology of Andhra Pradesh state.

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RESEARCH ARTICLE

Yellow Nana–A Dwarf Pomegranate (*Punica granatum*) Variety for Ornamental Purposes

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Abstract

The genus *Punica* to which pomegranate belongs also has a miniature pomegranate 'Nana', which produces orange red flowers with pink pigmentation in the new foliage and it is often used for decorative purposes. We developed a yellow variant of 'Nana' by introgressing the gene for yellow pigment from edible 'Kabul Yellow' variety. "Yellow Nana" was evaluated for 46 characters, including morphological and physico-chemical traits in comparison with Nana. The results showed the morphological similarity between 'Yellow Nana' and 'Nana' except for flower, fruit and aril colour. Most distinguishable characters of the 'Yellow Nana' are the dwarf plant stature (0.8 m), miniature leaves, profuse yellow flowers with white petals, small yellow fruits (9.45 g weight) with tiny light yellow highly acidic arils (5.19%) and hard seeds in comparison to 'Nana'. 'Yellow Nana' can grow as a new ornamental pomegranate variety as it is phenotypically similar to 'Nana' variety except for flower and foliage colour.

Keywords: Morphological traits, Ornamental pomegranate, Physico-chemical traits, Variety

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Introduction

Pomegranate (*Punica granatum* L.) is known to be one of the most versatile commercial fruit crops widely grown for fresh consumption as well as processing purposes (Pal *et al*., 2014). It belongs to 'Lythraceae' family with two species in the genus *Punica* (Graham *et al*., 2005). *P. granatum* L. is the only cultivated species found in the genus *Punica* with two sub-species *viz.,* chlorocarpa (green ovary) and porphyrocarpa (red ovary) (Rana *et al*., 2010). *P. granatum* L. has the wild ancestor of *P. protopunica* Balf. found endemic to the remote archipelago of Socotra, Yemen (Shilkina, 1973; Guerrero-Solano *et al.,* 2020). Wild forms of pomegranate are also known to grow in Iran, Afghanistan, Pakistan, Turkmenistan and North India (Holland *et al*., 2009).

In addition to cultivated and wild pomegranates, two ornamental types *viz.,* 'Double flower' and dwarf 'Nana', have also been reported (Jalikop, 2010). 'Double flower' types produce large, attractive double flowers wherein numerous stamens are modified into petals, resulting in no fruit set or few fruits. 'Nana' is a natural dwarf pomegranate which grows to a height of 50-70 cm, produces miniature leaves, flowers, fruits and arils (Nath and Randhawa, 1959; El-Moghazy *et al*., 2015). Several attempts have been made to exploit the ornamental pomegranates in breeding for moisture stress, bacterial blight resistance, understanding the genetics of fruit acidity, and

developing new ornamental pomegranates (Jalikop *et al*., 2003; Jalikop *et al*., 2006).

The commercially grown pomegranate develops into a tall shrub, bearing large red flowers and sizable fruits with red pigmentation. In contrast, 'Nana' is a miniature bonsai-like compact shrub and grows to a height of about 2 feet. It is regarded as an ornamental plant and grows well as a potted plant. The tiny flowers, fruits and petiole base of Nana have a red pigment, as in several cultivated pomegranates. 'Kabul Yellow' is a rare pomegranate variety that has yellow pigmentation in flowers, fruits, and foliage. With the objective of developing a yellow coloured miniature pomegranate, 'Nana' and 'Kabul Yellow' were hybridized and further by repeated backcrossing to 'Nana', segregants akin to 'Nana' with yellow pigment were isolated. The selected yellow plants were multiplied and evaluated for their performance under field conditions.

Materials and Methods

To develop a yellow miniature ornamental pomegranate, a crossing programme involving diverse cultivars like 'Nana' and 'Kabul Yellow' was carried out in 2009. Red pigment was found to be dominant over yellow, which is governed by a single recessive gene (rr) (Jalikop and Sampath Kumar, 1989). To transfer yellow pigmentation, two populations were developed: (1) (KY $\times$ N) and (2) [(KY $\times$ N) $\times$ N]-F_2 $\times$ [KY $\times$ N]-F_2 .The (KY $\times$ N) cross was preferred due to better fruit set, higher seed count, and improved germination. All 90 F_1 seedlings from this cross were pink, confirming the dominance of pink over yellow. Fifteen hybrids were field-planted, exhibiting compact canopies with normal flowering and fruiting. Poor germination in selfed (2.27%) and open-pollinated (4.12%) seeds limited large F_2 population development. However, hybrid 43H showed better germination (18.04%). Its F_2 progeny segregated in a 75:19 pink to yellow ratio, fitting a 3:1 pattern ($\chi^2 = 1.149$), indicating a single recessive gene (rr) controls yellow pigmentation. Of 94 F_2 seedlings, 20 resembled 'N' in plant habit. To address the issue of poor seed germination and enhance the survival rate of progenies in subsequent generations, the 'Ganesh' variety, known for its excellent seed germination, was incorporated into the breeding program. The following crosses were developed: (1) [(KY $\times$ N) $\times$ F_1] $\times$ [(G $\times$ N) $\times$ N]-F_4 and (2) N X {[(KY $\times$ N) $\times$ F_1] $\times$ [(G $\times$ N) $\times$ N]-F_2}- F_2. As a result, several miniature yellow seedlings resembling the 'Nana' variety were obtained, and the most fertile genotypes among them were selected for further evaluation.

The field evaluation was carried out at ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra (17°68' N latitude, 75°91' E longitude; 483.5 m above sea level) for four consecutive years (2019-2024). In 2017, 'Nana' and 'Yellow Nana' were planted at 4.5 m $\times$ 3 m and followed the standard management practices including timely pruning, training, weeding, irrigation, fertilizer application etc.

In total, 46 characters, including 10 qualitative and 36 quantitative traits, were recorded in the new variety ('Yellow Nana') along with its parental check variety ('Nana'). The DUS characters were recorded as per the DUS descriptor of PPV&FRA, New Delhi (https://plantauthority.gov.in/sites/ default/files/ dpomegranate.pdf). For the morphological characterization, plant material was randomly sampled from 10 plants. Fifteen mature leaves, 15 hermaphrodite flowers at full bloom, and 30 fruits at maturity were analysed. Tree height, spread, linear dimensions and weight were measured using measuring tape, digital Vernier Caliper, and precision electronic balance. Volume was determined by the liquid displacement method and expressed in ml. Total soluble solids (°Brix) of the arils juice was recorded using a digital refractometer. The titratable acidity of arils juice was measured by titration against 0.1 N NaOH solution using phenolphthalein indicator. Fruit and aril colour were measured by using a colorimeter and expressed in L*, a*, and b* coordinates. The seed hardness (N) was measured using a texture analyzer. Fruit juiciness was estimated on volume/weight (ml/g) basis and expressed in percentage. All seeds were extracted from each fruit and shade-dried under room conditions before taking the count and weight (g) data. Qualitative traits like tree foliage density, precocity, leaf blade shape, leaf apex shape, petiole anthocyanin colour, calyx colour, corolla colour, corolla type, crown neck, nipple/fin were measured on the selected plants of each variety.

The pooled mean data of the recorded quantitative traits were analysed for various descriptive statistical parameters in MS Excel 2016 software. Two-sample T-test was carried out to determine the statistical differences between the genotypes by using Web Agri Stat Package (WASP 2.0) developed by Jangam and Thali (2004).

Results and Discussion

'Nana', a natural dwarf pomegranate, differs from the edible variety for its dwarf plant stature, miniature leaves, tiny flowers and non-edible fruits. In warm-temperate climates, it is deciduous, but indoors and in the tropics, it behaves as an evergreen (El-Moghaz *et al*.,

2015; Sánchez-Hernández, 2022). Earlier gene exchange between cultivated (Ganesh) and decorative pomegranates (Nana, Double flower) has been attempted for bacterial blight resistance and drought resistance. Wherein 'Nana', 'Ganesh × Nana' and '(Ganesh × Nana) × Daru' showed a high level of resistance to bacterial blight disease. For drought resistance based on cell membrane stability, 'Nana' has shown as low as 1% in contrast to 16% in Ganesh. Hybrids involving Double flower with Ganesh (5.1%), Kabul Yellow (5.73%) and Nana (4.79%) also had very low CMS values (Jalikop *et al*., 2003). Inheritance of fruit acidity in pomegranate was studied in three sweet and three sour varieties and their progenies. The F_1 and F_2 data of 'Ganesh × Nana' showed that fruit acidity is monogenically controlled, and the sour nature is dominant over the sweet. (Ganesh × Nana) × Daru crossed progenies have produced acidity as high as 71.2 g/l due to the influence of modifying genes derived from the two acidic varieties 'Nana' and 'Daru' (Jalikop, 2007).

In this study, we have compared the performance of selected 'Yellow Nana' with the well-known ornamental variety 'Nana'. Both varieties were found to exhibit a similar phenotype of medium tree foliage density, early flowering, lanceolate leaf blade shape, obtuse leaf apex shape, low petiole anthocyanin colouration, single corolla type, fruits with crown neck and lack of nipple/fin in fruits, traits as per PPV&FRA guidelines. Non-significant similarities were also observed between 'Yellow Nana' and 'Nana' for most of the assessed characters. The most significant distinctive traits were found to be flower colour, fruit colour and aril colour. Wherein, 'Yellow Nana' showed the phenotype of 'Yellow calyx', 'White corolla' and 'Yellow' fruits with light yellow arils similar to 'Kabul Yellow'. Tree height of 'Yellow Nana' (0.80 m) was on par with 'Nana' (0.60 m) and was early (91 days), produced minute leaves, tiny yellow flowers, small fruits (9.45 g) that contained acidic (5.19%) hard seeded arils with very tiny and shorter (0.98 g) seeds (Table 1, Fig. 1). Similar, evaluation and morphological characterization using DUS test guidelines has been carried in different crops like Sweet Cherry (Dangi *et al*., 2021), Mango (Kumar *et al*., 2023) etc. In pomegranate, such morphological and physico-chemical comparison studies have been largely carried out for edible commercial varieties (Shilpa *et al*., 2024). Few molecular studies have been carried out using ornamental pomegranates, like understanding the genomic variation and phenotype in single and double-petal varieties (Huo *et al.,* 2023), and single-nucleotide polymorphism markers from de-novo assembly of the two *P. granatum* accessions *viz.,* 'Nana' (P.G.233–244) and 'Black' (P.G.127–28) transcriptome to reveal the genetic diversity in the 105 ARO germplasm collection (Ophir *et al*., 2014).

'Yellow Nana' was distinct from cv. 'Nana' for its flower colour, fruit colour, and aril colour. 'Yellow Nana' described in this paper can go as a new ornamental variety with decorative value, which can be grown in limited space and in pots as it produces attractive yellow flowers, foliage and tiny fruits, and resembles the established pink pigmented ornamental 'Nana' in respect of miniature plant habit. Large-scale multiplication and selling of plants of this variety will generate income for nurserymen, indoor gardeners and other stakeholders.

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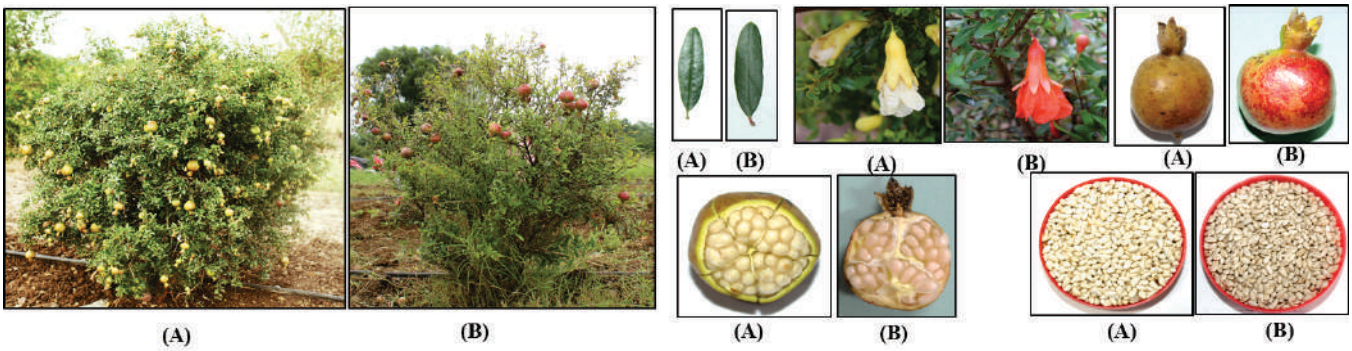

Fig. 1: Morphological features of pomegranate varieties (A) new dwarf variety "Yellow Nana" (B) parental check variety "Nana" for selected DUS characters.

Table 1: Morphological, physico-chemical characteristics of identified ornamental variety (Yellow Nana) and parental check variety (Nana)

Character	Yellow Nana					Nana					T-Statistic
	Mean	Range	SD	CV%	SE m	Mean	Range	SD	CV%	SEm	
Tree height(m)	0.80	0.58-0.97	0.12	15.21	0.04	0.60	0.46-0.85	0.10	17.23	0.03	4.30**
Tree spread(m)	0.61	0.45-0.74	0.11	17.81	0.03	0.40	0.25-0.625	0.10	25.34	0.03	4.85**
Shoot thorniness	5.41	3.07-11.80	2.75	50.87	0.80	5.20	3.33-8	1.42	27.27	0.41	0.24
Leaf blade length(cm)	2.40	1.95-3.07	0.38	15.70	0.11	2.19	1.70-2.87	0.40	18.17	0.12	1.32
Leaf blade width(cm)	0.65	0.51-0.77	0.09	13.29	0.02	0.64	0.43-0.90	0.15	22.84	0.04	0.21
Petiole length(mm)	2.07	0.96-2.68	0.52	24.92	0.15	2.42	1.67-3.3	0.55	22.78	0.16	-1.60
Petiole width(mm)	0.45	0.02-0.81	0.22	47.96	0.06	0.51	0.29-0.92	0.22	43.95	0.06	-0.60
Calyx length(mm)	21.32	17.56-26.81	2.69	12.62	0.78	20.24	12.43-24.87	3.49	17.24	1.01	0.84
Calyx width(mm)	6.95	4.23-9.58	1.52	21.88	0.44	6.74	4.71-10.54	1.55	22.96	0.45	0.33
Petal length(mm)	15.91	9.74-18.48	2.96	18.61	0.85	15.46	12.17-20.21	2.48	16.02	0.71	0.41
Petal width(mm)	10.92	6.85-13.65	2.31	21.15	0.67	10.45	6.72-13.67	2.80	26.76	0.81	0.45
No of fruits/plant	38.20	20-75.40	16.62	43.50	4.80	39.32	18.6-72	17.71	45.03	5.11	-0.16
Fruit weight(g)	9.45	3.86-19.96	4.03	42.68	1.16	13.46	9.36-22.75	3.36	24.95	0.97	-2.64*
Fruit volume	5.68	1.40-15.20	3.32	58.50	0.96	9.74	7.00-15.9	2.43	24.96	0.70	-3.41**
Fruit density	1.59	1.33-1.84	0.18	11.40	0.05	1.44	1.17-1.75	0.19	13.37	0.06	1.92
Fruit length (cm)	2.50	1.95-3.35	0.38	15.24	0.11	2.86	2.44-3.36	0.30	10.63	0.09	-2.54*
Fruit diameter (cm)	2.46	1.96-3.28	0.35	14.25	0.10	2.87	2.61-3.43	0.20	7.08	0.06	-3.49**
Crown length (cm)	1.14	1.02-1.31	0.12	10.81	0.04	1.21	0.95-1.48	0.16	13.20	0.05	-1.25
Crown width (cm)	0.72	0.56-0.88	0.09	12.99	0.03	0.87	0.75-1.01	0.09	9.75	0.02	-4.18**
Fruit shape (Ratio of longitudinal and lateral axes)	1.08	1.03-1.14	0.03	3.04	0.01	1.10	1.03-1.40	0.11	10.26	0.03	-0.84
No of arils/fruit	99.61	54.25-216.25	48.51	48.70	14.00	114.48	45.03-215.66	44.82	39.15	12.94	-0.78
Aril %	51.41	31.10-61.56	8.21	15.96	2.37	52.44	44.20-61.11	5.10	9.72	1.47	-0.37100
Aril weight (g)	4.50	3.20-6.31	0.91	20.27	0.26	4.16	3.14-5.55	0.79	18.98	0.23	0.97
Rind thickness (mm)	1.39	1.07-1.68	0.20	14.41	0.06	1.52	1.28-1.96	0.23	15.09	0.07	-1.47
TSS (^{0} Brix)	13.41	10.48-16.78	2.09	15.57	0.60	11.05	8.52-13.26	1.67	15.13	0.48	3.06**
Acidity %	5.19	2.42-6.72	1.57	30.34	0.45	4.73	2.48-.717	1.80	37.99	0.52	0.67
Aril length (mm)	5.93	5.08-6.97	0.54	9.09	0.16	6.38	5.55-7.19	0.53	8.27	0.15	-2.08*
Aril width (mm)	3.37	2.30-4.06	0.55	16.46	0.16	3.69	2.98-4.53	0.48	12.92	0.14	-1.54
Fruit Juiciness %(V/W)	16.53	8.80-39.31	8.21	49.66	2.37	10.65	2.45-20.11	5.25	49.27	1.52	2.09*
100 Seed weight(g)	0.98	0.74-1.48	0.26	26.81	0.08	1.01	0.59-1.26	0.22	21.36	0.06	-0.35
Seed length(mm)	5.13	4.63-5.60	0.31	5.98	0.09	4.85	4.51-5.30	0.31	6.35	0.09	2.21*
Seed width(mm)	2.09	0.82-3.00	0.79	37.56	0.23	1.97	1.12-3.2	0.85	43.11	0.25	0.36
Fruit colour(L*)	31.90	28.15-35.09	2.50	7.83	0.72	24.45	19.53-30.96	3.89	15.89	1.12	5.58**
a*	9.44	8.58-11.31	0.80	8.44	0.23	17.79	11.48-21.77	3.50	19.65	1.01	-8.06**
b*	22.99	18.55-26.42	2.74	11.92	0.79	20.59	15.80-27.97	3.04	14.74	0.88	2.03
Aril colour(L*)	52.38	47.08-55.09	2.73	5.20	0.79	55.94	50.36-59.25	2.85	5.09	0.82	-3.13**
a*	5.07	4.20-6.23	0.70	13.74	0.20	5.74	4.46-6.87	0.66	11.44	0.19	-2.42*
b*	19.92	17.38-22.32	1.50	7.51	0.43	23.42	19.65-25.65	2.02	8.64	0.58	-4.82**
Seed hardiness(N)	59.42	51.28-69.31	6.51	10.95	1.88	57.28	50.76-71.52	6.69	11.68	1.93	0.79
Fruit maturity(days after anthesis)	91.42	77-110	10.47	11.46	3.02	96.83	85-120	9.59	9.90	2.77	-1.32

*significant at 0.05 level of significance; ** significant at both 0.05 and 0.01 levels of significance.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

All data are included in the manuscript.

Authors' Contributions

PS, SHJ, KDB, RK and RSP Conceptualization, methodology, investigation, analysis, interpretation of data; SHJ and RK sharing of experimental material; SP and RSP drafted the work; SP, GAR and TD Data collection, compilation; SHJ, KDB and RAM revised and edited the work; RAM Supervision, funding acquisition and final approval.

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RESEARCH ARTICLE

Role of Macrosclereid Length in Seed Hardness and Dormancy in *Vigna* Species

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Abstract

Pulses are an important and affordable source of dietary protein, and the genus *Vigna* includes species with high agronomic and nutritional value. Seed dormancy, mainly controlled by seed coat hardness, is a key trait that affects germination, crop establishment, and management of genetic resources. In this study, we explored how macrosclereid length influences hard-seededness (Physical dormancy) in six *Vigna* species representing different levels of domestication: domesticated (*V. mungo*, *V. radiata*), semi-domesticated (*V. stipulacea*), and wild relatives (*V. sublobata*, *V. silvestris*, *V. setulosa*). We studied nineteen accessions for seed coat anatomy using a light microscope, and the data were analysed through ANOVA, Duncan's test, and correlation analyses. We found a clear domestication-related pattern: domesticated species had smoother testa with shorter macrosclereids, wild species showed rough, thick-walled testa with longer macrosclereids, and the semi-domesticated species was intermediate. Macrosclereid length and seed hardness were negatively associated with germination. These results shed light on the structural basis of dormancy and provide guidance for germplasm conservation and breeding for improved seed permeability in *Vigna*.

Keywords: Anatomy, Domestication, Macrosclereid length, Physical dormancy, *Vigna*

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Introduction

The Fabaceae is one of the largest and most diverse families of flowering plants, comprising more than 770 genera and about 20,000 species, and representing nearly 5% of all known plant diversity (Lewis *et al.,* 2005). Members of this family hold great agronomic and ecological importance as they provide protein-rich seeds for human consumption, forage for livestock, and green manure that improves soil fertility. Their symbiotic association with nitrogen-fixing bacteria such as *Rhizobium* and *Bradyrhizobium* contributes to soil health and supports sustainable agricultural systems (Graham and Vance, 2003; Sprent, 2009; Peoples *et al.,* 2009).

Within Fabaceae, the genus *Vigna* is pantropical and highly diverse, comprising more than 200 species distributed mainly across Asia and Africa (Pratap *et al.,* 2014; Tripathi *et al.,* 2019). India serves as a major centre of origin and diversification for several cultivated taxa, including mungbean (*V. radiata*), urdbean (*V. mungo*), mothbean (*V. aconitifolia*), and rice bean (*V. umbellata*) (Bisht and Singh, 2005; Pradheep *et al.,* 2014). These crops are valued for their short duration, adaptability to marginal environments, and contribution to food and nutritional security, particularly in rainfed regions (Ali and

Gupta, 2012). However, the effective utilization of *Vigna* germplasm is constrained by seed dormancy, especially in wild and semi-domesticated species.

Seed dormancy in legumes is predominantly physical, caused by a water-impermeable seed coat that prevents imbibition even under favourable conditions. While this trait enhances survival and persistence under natural habitats, it is undesirable in cultivation due to its adverse effects on germination and crop establishment (Singh *et al.,* 2020; Soltani *et al.,* 2021). The impermeability of legume seed coat is mainly associated with the palisade or macrosclereid layer, which is reinforced with cuticular deposits of lignin, suberin, and waxes (Cechová *et al.,* 2017; Hradilová *et al.,* 2017; Janská *et al.,* 2019). Variations in macrosclereid structure, seed coat surface morphology, and hilum characteristics are known to influence permeability and dormancy expression (Ignacimuthu and Babu, 1985; Umdale *et al.,* 2017; Paul *et al.,* 2024).

Despite advances in understanding the anatomical and physiological aspects of seed dormancy in legumes, comparative studies linking macrosclereid cell characteristics with hard-seededness in *Vigna* remain limited. Understanding these structural differences is crucial for explaining interspecific variation in seed hardness and dormancy. The present study was therefore undertaken to examine the relationship between macrosclereid length and seed hardness among selected domesticated, semi-domesticated, and wild *Vigna* species.

Materials and Methods

A total of 19 accessions representing six species of the genus *Vigna* were selected for the study, including domesticated species (*V. mungo*, *V. radiata*), a semi-domesticated species (*V. stipulacea*), and wild species (*V. sublobata*, *V. silvestris*, *V. setulosa*). The accessions were obtained from the Division of Germplasm Evaluation, ICAR-NBPGR, New Delhi. Detailed information on the species, accession numbers, and passport data is provided in Table 1.

Histology of the seed coat

Histological studies were carried out to observe the internal structure of the seed coat and its relationship with physical dormancy in *Vigna* species. Mature, well-developed seeds were cleaned thoroughly to remove any surface impurities and then soaked in distilled water for 24 hours to soften the outer layers. The seed coats, including the hilum and micropylar regions, were carefully dissected under a stereomicroscope to ensure that the tissues remained intact for microscopic observation.

The excised seed coats were fixed in a formalin acetic acid alcohol (FAA) solution composed of 50% ethanol, 5% acetic acid, and 10% formalin, and kept for

Table 1: Passport Data of the *Vigna* species accessions

S.no	Species	Acc.no	Status	District	State
1.	*V. mungo*	IC650620	Domesticated	Kanpur	Uttar Pradesh
2.	*V. mungo*	IC651317	Domesticated	Sehore	Madhya Pradesh
3.	*V. mungo*	IC652065	Domesticated	Guntur	Andhra Pradesh
4.	*V. radiata*	IC650613	Domesticated	New Delhi	Delhi
5.	*V. radiata*	IC651263	Domesticated	Sehore	Madhya Pradesh
6.	*V. radiata*	IC652064	Domesticated	Guntur	Andhra Pradesh
7.	*V. stipulacea*	IC550532	Semi-domesticated	Vizianagaram	Andhra Pradesh
8.	*V. stipulacea*	IC622867	Semi domesticated	Tiruchirappalli	Tamil Nadu
9.	*V. stipulacea*	IC651911	Semi-domesticated	Bhubaneswar	Odisha
10.	*V. sublobata*	IC202643	wild	Thrissur	Kerala
11.	*V. sublobata*	IC331468	Wild	Jabalpur	Madhya Pradesh
12.	*V. sublobata*	IC322299	Wild	Thrissur	Kerala
13.	*V. sublobata*	IC406507	Wild	Palakkad	Kerala
14.	*V. silvestris*	IC585931	Wild	Mumbai	Maharashtra
15.	*V. silvestris*	IC618518	Wild	South Goa	Goa
16.	*V. silvestris*	IC622873	Wild	South Goa	Goa
17.	*V. silvestris*	IC622877	Wild	Uttara Kannada	Karnataka
18.	*V. setulosa*	IC251419	Wild	Unknown	Unknown
19.	*V. setulosa*	IC252003	Wild	Unknown	Unknown

24 hours at room temperature. After fixation, the samples were rinsed in 70% ethanol to remove residual fixative and stored at 4°C until further processing. Dehydration was carried out through a graded tertiary butyl alcohol (TBA) series, followed by infiltration and embedding in paraffin wax maintained at 58–60°C. Thin transverse sections, 10 µm thick, were obtained using a rotary microtome and mounted on adhesive-coated glass slides to prevent detachment during staining.

For histochemical staining, Toluidine Blue O (Sakai, 1973) was used to distinguish lignified tissues, while Ruthenium Red (Soukup, 2014) was employed to detect mucilage and pectic substances. The stained sections were mounted in DPX and examined under a compound microscope, Leica DM500, at magnifications ranging from 10X to 40X. Photomicrographs were captured and calibrated using a stage micrometre, and the measurements of the palisade layer and macrosclereid cells were taken with ImageJ software. These observations provided insights into the structural variations in the seed coat contributing to dormancy expression among *Vigna* species.

Measurement of seed weight

Randomly select ten mature, dry seeds from each accession. Weigh the seeds using a precision analytical balance (±0.001 g). Calculate the average seed weight (mg/seed).

Measurement of seed hardness

The Seed Hardness Machine, Parisa technology device, is used to determine the resistance of a seed to deformation or fracture. Select representative, clean seeds from each accession. Place individual seeds in the hardness machine. Record the force in Newton (N) required to crack or break each seed. Mean values calculated from the three replicates for each accession.

Measurement of different parameters of the seed-by-seed analyser

Seed morphology and colour characteristics were analysed using an automated seed image analysis system developed by Nachiket Kotwaliwale at the ICAR–Central Institute of Agricultural Engineering (CIAE), Bhopal. The system was operated with Smart Grain software (version 1.3). Before analysis, calibration was performed using standard reference scales for length and area to ensure measurement accuracy. Uniform and diffuse lighting was maintained during imaging to achieve consistent colour evaluation.

Clean and well-filled seeds were arranged on a flat, non-reflective surface to prevent glare and image distortion. Damaged or irregular seeds were excluded to maintain data reliability. High-resolution images were captured under controlled conditions, and the software automatically separated individual seeds from the background for measurement.

The software generated data for key geometric and colour parameters. Seed length was defined as the maximum dimension along the major axis, while seed breadth represented the maximum width along the minor axis. The extracted data were used for further analysis of morphological variation among *Vigna* species.

Measurement of hilum length and width with a vernier calliper

Hilum length and width were determined using a Vernier calliper to examine variation among seeds. Only mature, intact seeds with a clearly visible hilum were used for measurements. The calliper was calibrated to zero before each measurement. Hilum length was recorded from one end to the other, while width was measured at its widest point. For each accession, five seeds were measured, and the average values were calculated to ensure reliability and consistency of the data.

Data analysis

All recorded observations were analysed to assess variation among species and the relationships between traits. For each trait, descriptive statistics including mean, standard deviation, and coefficient of variation were calculated. One-way ANOVA was conducted to identify significant differences among species for morphological, physiological, and anatomical parameters. Pearson's correlation coefficients were used to explore the associations between seed size, hardness, germination, and macrosclereid length at significance levels of $P \leqslant 0.05$ and $P \leqslant 0.01$. Image-based measurements for anatomical study were obtained and verified using ImageJ (v1.54), and further statistical analyses were carried out using Microsoft Excel 2021 and SPSS 30.0 (IBM Corp., Armonk, USA).

Results

Analysis of variance (ANOVA) of seed traits for six Vigna species

The Analysis of Variance (ANOVA) shows that all seven seed parameters showed highly significant variation among the six *Vigna* species ($P \leqslant 0.05$ or $P \leqslant 0.01$), indicating that the species differ statistically in their seed characteristics (Table 2).

Table 2: Analysis of variance (ANOVA) of 7 seed traits for six *Vigna* species

Seed parameter	Sum of squares	Df	Mean square	F	Sig
Hundred seed weight (g)	87.92	5.00	17.58	58.40	0.00
Seed length (mm)	33.42	5.00	6.68	30.25	0.00
Seed width(mm)	14.71	5.00	2.94	19.80	0.00
Seed hilum length(mm)	4.87	5.00	0.97	10.91	0.00
Seed hilum width(mm)	1.49	5.00	0.30	5.20	0.00
Macroscleried length (µm)	42764.80	5.00	8552.96	30.69	0.00
Seed hardness (N)	100.05	5.00	20.01	42.46	0.00

Species-wise descriptive statistics

Seed traits varied notably among the six *Vigna* species studied (*V. mungo*, *V. radiata*, *V. setulosa*, *V. silvestris*, *V. stipulacea*, and *V. sublobata*) (Table 3).

The domesticated species, *V. mungo* (3.798 g) and *V. radiata* (3.402 g), had significantly larger seeds compared to the wild species, *V. setulosa* (0.732 g) and *V. sublobata* (0.956 g), while the semi-domesticated *V. stipulacea* (0.800 g) exhibited intermediate seed weight. Seed area, length, and width followed a similar trend, reflecting the influence of domestication on seed enlargement.

Dimensional parameters, including seed perimeter, equivalent diameter, axial and median lengths, and hilum size, were greater in cultivated species, with the longest hilum observed in *V. mungo* (2.166 mm). On the other hand, wild taxa displayed greater variability in these traits. Seed hardness was significantly lower in cultivated species (*V. mungo* 2.453; *V. radiata* 2.177) than in wild species (*V. setulosa* 6.108; *V. sublobata* 5.055). Similarly, macrosclereid length, which forms the palisade layer of the seed coat, was shortest in *V. mungo* (55.754 µm) and longest in *V. sublobata* (maximum 199.12 µm), indicating thicker seed coats in wild species.

Broadly, cultivated *Vigna* species produced larger, softer seeds with thinner coats, whereas wild species exhibited smaller, harder seeds with more robust palisade layers. These interspecific differences underscore the impact of domestication on seed morphology and dormancy-related traits.

Table 3: Description of variation in seed parameters among six *Vigna* species

Trait	*Vigna mungo*	*Vigna radiata*	*Vigna setulosa*	*Vigna silvestris*	*Vigna stipulacea*	*Vigna sublobata*
Hundred seed weight (g)						
Average	3.798	3.402	0.732	1.188	0.8	0.956
Max	4.32	4.8	0.85	1.71	0.85	1.59
Min	2.91	2.33	0.62	0.51	0.75	0.64
SD	0.642	1.038	0.101	0.415	0.034	0.367
CV (%)	16.908	30.516	13.869	34.923	4.239	38.392
Seed length (mm)						
Average	4.633	4.633	2.75	3.433	2.822	3.008
Max	5.2	5.6	2.9	4.1	3.1	3.6
Min	3.8	3.7	2.2	3	2.5	2.2
SD	0.561	0.7	0.274	0.35	0.186	0.507
CV (%)	12.113	15.108	9.959	10.191	6.576	16.858
Seed width (mm)						
Average	3.811	3.567	2.4	2.992	2.444	2.717
Max	4.3	4.3	2.8	3.6	2.8	3.4
Min	3	3	2.1	2.4	2.1	2.1
SD	0.434	0.406	0.276	0.396	0.27	0.432
CV (%)	11.395	11.389	11.487	13.253	11.036	15.917

Seed Hilum length (mm)						
Average	2.166	1.644	1.37	1.893	1.34	1.458
Max	2.61	2.01	2.17	2.19	1.49	2.04
Min	1.67	1.31	0.52	1.36	1.27	1.05
SD	0.332	0.254	0.54	0.269	0.065	0.282
CV (%)	15.321	15.426	39.394	14.202	4.851	19.329
Seed Hilum width (mm)						
Average	0.997	0.684	0.578	0.99	0.682	0.678
Max	1.34	1.05	1.07	1.31	0.9	1.67
Min	0.81	0.44	0.32	0.52	0.57	0.36
SD	0.147	0.171	0.271	0.256	0.107	0.348
CV (%)	14.738	25.021	46.902	25.828	15.749	51.26
Macroscleried length (µm)						
Average	55.754	83.099	111.227	115.323	83.081	136.998
Max	82.17	95.51	122.29	150.98	93.45	199.12
Min	41.06	77.93	103.26	97.5	64.97	116.23
SD	19.432	5.673	6.846	16.742	9.372	25.07
CV (%)	34.853	6.827	6.155	14.517	11.28	18.3
Seed hardness (N)						
Average	2.453	2.177	6.108	4.867	4.262	5.055
Max	2.922	2.86	7.877	6.644	4.577	7.185
Min	1.616	1.749	5.107	4.125	4.01	4.028
SD	0.486	0.36	1.101	0.708	0.203	0.915
CV (%)	19.797	16.545	18.021	14.546	4.761	18.104

Macrosclereid length

The macrosclereid length exhibited substantial interspecific variation among the six *Vigna* species evaluated (Table 4; Fig. 1). In the cultivated species *V. mungo* and *V. radiata*, the macrosclereid cells were relatively shorter, ranging from 41.06 µm to 82.17 µm and 77.93 µm to 95.51 µm, with mean lengths of 55.75 ± 19.43 µm and 83.10 ± 5.67 µm, respectively.

Conversely, the wild species exhibited considerably higher macrosclereid dimensions. *V. setulosa* and *V. silvestris* recorded mean lengths of 111.23 ± 6.85 µm and 115.32 ± 16.74 µm, respectively, with maximum values reaching 122.29 µm and 150.98 µm. While the semi-domesticated species *V. stipulacea* displayed intermediate values (mean 83.08 ± 9.37 µm, range 64.97–93.45 µm), *V. sublobata* possessed the longest macrosclereid cells, varying from 116.23 µm to 199.12 µm with a mean of 136.99 ± 25.07 µm.

The coefficient of variation (CV) was lowest in *V. radiata* (6.83%) and *V. setulosa* (6.16%), indicating greater uniformity within the cultivated and wild accessions, while *V. mungo* showed the highest variability (34.85%). A significant trend of increasing macrosclereid length was evident from cultivated to wild taxa, suggesting progressive enhancement of palisade cell elongation in wild *Vigna* species.

Table 4: Duncan's multiple range test of seed parameters across species

Species	Hundred seed weight	Seed length	Seed width	Hilum length	Hilum width	Macroscleried length	Seed hardness
V. mungo	3.798± 0.21 b	4.63±0.19c	3.811±0.14c	2.17±0.11c	0.997±0.05b	55.754±6.48a	2.453±0.16a
V. radiata	3.402± 0.35 b	4.63±0.23c	3.567±0.14c	1.64±0.08ab	0.684±0.06a	83.099±1.89b	2.177±0.12a
V. sublobata	0.956± 0.11 a	3.00±0.15ab	2.717±0.12ab	1.46±0.08a	0.678±0.10a	136.998±7.24d	5.055±0.26c
V. silvestris	1.188± 0.12 a	3.43±0.10b	2.992±0.11b	1.89±0.08bc	0.990±0.07b	115.323±4.83c	4.867±0.20bc
V. setulosa	0.732 ± 0.04 a	2.75±0.11a	2.400±0.11a	1.37±0.22a	0.578±0.11a	111.227±2.79c	6.108±0.45d
V. stipulacea	0.800 ± 0.01 a	2.822±0.6a	2.444±0.09a	1.34±0.02a	0.682±0.04a	83.081±3.12b	4.262±0.07b

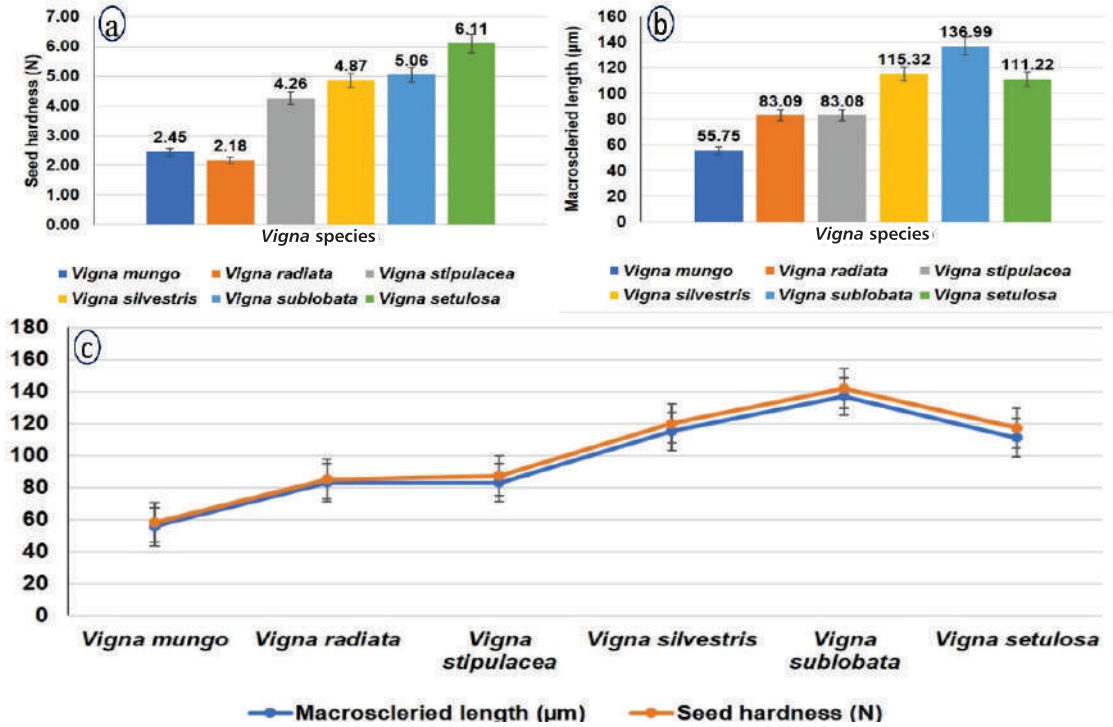

Fig. 1: (a) Seed hardness across *Vigna* species (N), (b) Macrosclereid length across *Vigna* species (µm), (c) Relationship between macrosclereid length and seed hardness across *Vigna* species.

In general, species with higher macrosclereid length (⩾120 µm), notably *V. sublobata* and *V. silvestris*, are likely to exhibit stronger physical dormancy compared to cultivated species such as *V. mungo* and *V. radiata*. The distribution and interspecific differences in macrosclereid length are illustrated in Fig. 1.

Seed hardness

Seed hardness varied widely among the six *Vigna* species studied, showing a clear interspecific pattern in seed coat strength. Overall values ranged from 1.62 to 7.88 N, reflecting considerable diversity in the mechanical resistance of the seed coat. The cultivated species showed lower hardness compared to their wild relatives. In *V. radiata*, seed hardness ranged from 1.75 to 2.86 N with a mean of 2.18 ± 0.36 N and a coefficient of variation of 16.55%. Similarly, *V. mungo* recorded values between 1.62 and 2.92 N, with a mean of 2.45 ± 0.49 N and a coefficient of variation of 19.80%. These two cultivated species formed the low-hardness group, indicating thinner and more permeable seed coats.

Among the wild species, *V. setulosa* showed the greatest hardness, ranging from 5.11 to 7.88 N with a mean of 6.11 ± 1.10 N and a coefficient of variation of 18.02% *V. silvestris* and *V. sublobata* exhibited intermediate to high values, with means of 4.87 ± 0.71 N and 5.06 ± 0.92 N, respectively. The semi-domesticated *V.stipulacea* occupied a moderate position, showing hardness values between 4.01 and 4.58 N with a mean of 4.26 ± 0.20 N and the lowest variability among species, with a coefficient of variation of 4.76%.

Across species, *V. stipulacea* showed the narrowest range of hardness values, suggesting a uniform seed coat texture, while *V. setulosa* displayed the widest range, indicating greater heterogeneity in seed coat resistance. When grouped by relative hardness, *V. mungo* and *V. radiata* represented the low class, *V. stipulacea* the moderate class, and *V. silvestris*, *V. sublobata*, and *V. setulosa* the high-hardness class. The general trend indicated a gradual increase in seed hardness from cultivated to wild taxa, reflecting progressive thickening and lignification of the seed coat during evolution. The interspecific differences in seed hardness are shown in Fig. 1.

Correlation analysis of seed traits

The correlation analysis revealed clear associations among the measured seed traits (Table 5). Hundred-seed weight showed a strong positive relationship with seed length (r = 0.893**), seed width (r = 0.815**), and germination percentage (r = 0.781**), indicating that larger seeds tended to germinate more efficiently. Conversely, hundred-seed weight was negatively correlated with macrosclereid length (r = –0.581**) and

Table 5: Correlation among seed traits in *Vigna* species

Seed trait	Hundred seed weight	Seed length	Seed width	Hilum length	Hilum width	Macroscleried length	Seed hardness	Germi-nation
Hundred seed weight	1							
Seed length	0.893**	1						
Seed width	0.815**	0.913**	1					
Hilum length	0.483**	0.484**	0.574**	1				
Hilum width	0.251	0.325*	0.382**	0.669**	1			
Macroscleried length	-0.581**	-0.516**	-0.464**	-0.374**	-0.165	1		
Seed hardness	-0.751**	-0.782**	-0.703**	-0.321*	-0.162	0.640**	1	
Germination	0.781**	0.682**	.609**	0.192		0.0185	-0.508**	-0.795**1

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed)

seed hardness (r = –0.751**), indicating that smaller, harder seeds tend to be lighter.

Seed length and width were closely related (r = 0.913**), and both showed positive associations with germination, confirming that seed size influences germination performance. Hilum length correlated positively with hilum width (r = 0.669**) and moderately with seed size traits, though the relationships were comparatively weaker.

Macrosclereid length showed significant negative correlations with seed size parameters, including hundred-seed weight, seed length, and seed width, but had a strong positive relationship with seed hardness (r = 0.640**). Similarly, seed hardness was negatively correlated with seed length (r = –0.782**), seed width (r = –0.703**), hundred-seed weight (r = –0.751**), and germination (r = –0.795**), confirming that seeds with thicker macrosclereid layers and greater hardness tend to be smaller and less permeable.

Relationship between Macrosclereid length and seed hardness

Significant variation was observed in macrosclereid length and seed hardness among the six *Vigna* species studied (Fig. 1). Domesticated species such as *V. mungo* (55.75 µm) and *V. radiata* (83.09 µm) had significantly shorter macrosclereid cells compared to wild species like *V. silvestris* (115.32 µm), *V. sublobata* (136.99 µm), and *V. setulosa* (111.22 µm). The semi-domesticated *V. stipulacea* recorded an intermediate value (83.08 µm). Seed hardness followed a similar pattern. The lowest values were recorded in cultivar species like *V. radiata* (2.18 N) and *V. mungo* (2.45 N), while the wild species like *V. silvestris* (4.87 N), *V. sublobata* (5.06 N), and *V. setulosa* (6.11 N) showed much higher seed hardness. The semi-domesticated species *V. stipulacea* showed intermediate hardness (4.26 N). The strong positive correlation between macrosclereid length and seed hardness (Fig. 1), with both parameters increasing progressively from domesticated to wild types. *V. sublobata* exhibited the highest values for both macrosclereid length (136.99 µm) and seed hardness (5.06 N), suggesting that thicker macrosclereid layers contribute to seed coat strength and dormancy in wild species, while domesticated species have evolved thinner, softer seed coats promoting faster germination.

Discussion

The six *Vigna* species (*V. mungo*, *V. radiata*, *V. setulosa*, *V. silvestris*, *V. stipulacea*, and *V. sublobata*) showed considerable variation in seed morphology, structure, and hardness, reflecting their differing domestication levels, ecological adaptations, and phylogenetic relationships. Analysis of Variance (ANOVA) revealed that all 7 seed traits differed significantly among the species ($P \leqslant 0.05$ or $P \leqslant 0.01$), indicating clear statistical divergence (Table 1). Duncan's multiple range test further confirmed distinct groupings for each trait (Table 4), highlighting pronounced interspecific differences.

Seed traits clearly distinguished domesticated and wild *Vigna* species. Cultivated species, *V. mungo* and *V. radiata*, produced larger seeds with higher 100-seed weight, greater area, length, and width, reflecting human selection for traits that enhance seedling vigour and crop productivity (Gepts, 2004; Fuller, 2007; Zohary *et al.,* 2012;). In contrast, wild species such as *V. setulosa*, *V. stipulacea*, and *V. sublobata* had smaller, compact seeds, which likely support persistence in natural

habitats through efficient dispersal and dormancy (Koinange *et al.,* 1996; Tomooka *et al.,* 2002)

Seed hardness and dormancy characteristics further emphasised these differences between cultivated and wild species. Wild species exhibited harder seeds and longer macrosclereid cells, forming tougher seed coats that delay germination until environmental conditions are favourable. By contrast, cultivated species had softer seeds and shorter palisade layers, enabling rapid and uniform germination under agricultural management (Rolston, 1978; Smýkal *et al.,* 2014; Dissanayake *et al.,* 2016). Notably, the high variability in seed hardness in *V. sublobata* indicates untapped genetic potential for breeding programs targeting dormancy and stress resilience. Together, these findings reveal the domestication syndrome in *Vigna*, where seed size, shape, and dormancy traits have evolved under human selection, while wild species retain compact, robust seeds adapted to natural environments. Wild *Vigna* species thus represent valuable genetic resources for breeding programs aiming to combine agronomic performance with adaptive resilience (Fuller & Allaby, 2009; Gopinath *et al.,* 2021).

Correlation analysis revealed clear relationships among seed morphological and physiological traits in *Vigna* species. Larger seeds, indicated by higher hundred-seed weight, were strongly associated with greater seed length (r = 0.893**) and width (r = 0.815**), as well as higher germination percentages (r = 0.781**). This shows that bigger seeds, with more nutrient reserves, support better seedling vigour and improved germination (Baskin & Baskin, 2014). Seed weight was negatively correlated with macrosclereid length (r = -0.581**) and seed hardness (r = -0.751**), reflecting a trade-off between seed size and dormancy. Wild species, with smaller, harder seeds and thicker palisade layers, showed stronger physical dormancy, whereas domesticated species had larger, softer seeds that germinate quickly and uniformly (Fuller & Allaby, 2009; Smýkal *et al.,* 2014).

Seed length and width were strongly correlated with each other (r = 0.913**) and positively linked to germination, while negatively associated with seed hardness and macrosclereid length. This highlights the central role of seed size in water uptake, mechanical resistance, and germination behaviour (Kumar *et al.,* 2011; Dissanayake *et al.,* 2016). Hilum dimensions, though moderately correlated with seed size, were not significantly related to germination or macrosclereid length, indicating that hilum grows proportionally with seed size but does not independently affect dormancy or water permeability (Srinivasan et al., 2007; Bewley *et al.,* 2013; Tripathi *et al.,* 2019; Gore *et al.,* 2025). Collectively, these results showed how domestication has shaped seed traits in *Vigna*, favouring larger seeds with reduced dormancy to improve germination and seedling vigour, while wild species retain smaller, harder seeds with structural adaptations that enhance survival in natural environments. Macrosclereid length was negatively correlated with hundred-seed weight, seed length, and seed width, but positively associated with seed hardness (r = 0.640**) and negatively with germination (r = -0.508**), highlighting its critical role in enforcing physical dormancy in wild *Vigna* species. As the main structural component of the palisade layer, macrosclereids determine seed coat thickness and impermeability, directly influencing germination potential (Tomooka *et al.,* 2002).

Seed hardness similarly acted as a negative regulator of germination. Strong negative correlations with seed size traits and germination (r = -0.795**) indicate that hard seeds restrict water uptake and delay germination, a trait advantageous for survival in natural ecosystems. The positive association between seed hardness and macrosclereid length underscores the anatomical basis of this physiological barrier (Maxted *et al.,* 2001; Smýkal *et al.,* 2014). In general, the correlation matrix shows the balance between seed enlargement, dormancy, and germination strategies in *Vigna* species. Domestication has favoured larger seeds with reduced hardness and shorter macrosclereids, promoting rapid and uniform germination. Conversely, wild species retain dormancy-enhancing traits, such as high hardness and thick palisade layers, which limit germination but enhance seed longevity and environmental persistence.

Conclusion

This study reveals a clear domestication gradient in seed dormancy across *Vigna*. Domesticated species (*V. mungo*, *V. radiata*) are non-dormant, with seed coat traits favouring rapid germination, while wild relatives (*V. sublobata*, *V. silvestris*, *V. setulosa*) maintain strong physical dormancy through enhanced anatomical features. The semi-domesticated *V. stipulacea* exhibits intermediate traits. Reduced hard-seededness in domesticated species is linked to shorter macrosclereids, smoother testa, and thinner cell walls, showing a domestication syndrome. These findings have practical applications, like wild species require species-specific pre-sowing treatments, such as mechanical scarification, for genebank regeneration, and understanding dormancy anatomy aids breeding

programs by enabling the use of wild germplasm without compromising germination. Future research should explore the genetic basis of macrosclereid development and cuticle deposition, as well as environmental influences on dormancy persistence and release.

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RESEARCH ARTICLE

Identification of Potential Bell Pepper Crosses with Improved Nutritional Traits Under Open Cultivation

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Abstract

A half diallel mating design was used to hybridize seven bell pepper inbred parental lines, yielding 21 F_1 hybrids, excluding reciprocals. The generated F_1 hybrids, parental lines, and commercial check hybrid were evaluated to investigate per se performance, combining ability effects, and the magnitude of heterosis over the better-parent and commercial check for quality traits such as ascorbic acid, total carotenoid, and antioxidant traits, using a randomised complete block design with three replications. It was inferred that hybrids that were superior based on per se performance involved good specific combiners, and their crosses were heterotic over commercial check. The present study also concluded the non-additive gene action, governing the nutritional traits in bell pepper.

Keywords: Ascorbic acid, Antioxidants, Bell pepper, Carotenoid, Nutritional trait, Phenols

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Introduction

Bell pepper (*Capsicum annuum* var. grossum) is a preferred vegetable among consumers worldwide owing to its unique flavour profile, which is a combination of six taste sensations, i.e. bitter, sweet, sour, salty, umami and pungent. In general, the purchasing decision of consumers depends on the appearance and attractiveness of fruit, but lately, consumers are also paying attention to flavour and nutritional value. Hence, scientists have started working on nutritional and nutraceutical characteristics as well. Bell pepper is considered as good source of antioxidants, flavonoids, phenolic acids and carotenoids. Depending on the colour of fruit, ascorbic acid ranges from 16.52 to 159.61 mg per 100 g of fresh bell pepper (Nerdy, 2018). Green bell pepper is an excellent source of ascorbic acid and a fair source of provitamin A and carotenoids (Sharma *et al.,* 2013). The red colour of bell pepper is due to the presence of carotenoid pigments that include β-carotene with provitamin A activity and oxygenated carotenoids such as capsanthin, capsorubin and cryptocapsin. It also contains enormous quantities of neutral phenolic compounds or flavonoids called quercetin, luteolin, and capsaicinoids. Bell pepper is believed to have medicinal properties and hence, recommended for the treatment of dropsy, colic, toothache and cholera. Phytochemicals present in bell pepper help in the prevention of oxidation of essential fats within cells of the brain that are

considered necessary for its optimal functioning (Aditika *et al.*, 2018).

In India, bell pepper cultivation is very popular in peri-urban production systems because of easy access to urban markets. It is basically a cool-season crop and the day temperature of less than 30°C and night temperature 16-18°C favours its optimum growth and development. The fruit is green when immature and turns red or yellow at maturity. It is a high-value and low-volume crop and is grown mainly under protected structures to avoid crop loss from various biotic and abiotic stresses. However, in places in and around Bangalore, Pune, mid-hills of Himachal Pradesh, where temperature does not go very high or low during the growing season, open cultivation of the crop is also practised. Definitely, the nutritional quality of fruits grown inside a protected structure is better compared to open cultivated one because of less exposure to biotic and abiotic stresses. Small to medium farmers of India cannot afford protected cultivation. Hence, this study was conducted to identify potential crosses that can produce better nutritional quality of fruits compared to their parents under open field conditions. One of the methods to improve nutritional parameters is heterosis breeding. Diallel analysis also allows the breeder to predict the performance of a cross in subsequent generations based on the behaviour of the F_1 itself. The diallel cross approach, being quick and efficient to estimate the combining ability, was adopted to investigate nutritional characters. Keeping these facts in view, the present investigation was planned and conducted to identify the superior crosses with respect to nutritional traits in bell pepper following a half-diallel mating design.

Materials and Methods

Experimental material, design and location

The present investigation was carried out during 2019-2020 for two seasons at farm block number 8 of the Division of Vegetable Crops, ICAR-Indian Institute of Horticulture Research, Bengaluru, India. The geographical location of the experimental farm is having a latitude of 13° 7' N and 77 ° 29' E. The 21 F_1 hybrids were developed by crossing seven diverse parents in a half diallel mating design from April to September 2019. Crossing was done by manual hand emasculation and pollination. Ripened cross-fertilised fruits were collected, and seed extraction was carried out. Evaluation of those hybrids was undertaken from October 2019 to March 2020. The experimental material consists of seven parents, namely, Arka Mohini (AM), Arka Basant (AB), Arka Gaurav (AG), Yolo Wonder (YW), Californina Wonder (CW), UHF-BP-4 and CW308. Twenty-one F_1 crosses and a check Indra (Syngenta Pvt. Ltd) were sown in pro-trays during October 2019, and healthy seedlings were transplanted in the field in November 2019 at a spacing of 60 cm x 30 cm in a randomized complete block design with three replications. Each replication had 25 plants in a row. Five randomly selected plants were tagged for observation in each replication. Mature green fruits were harvested from tagged plants and were analysed for ascorbic acid, total phenols, antioxidant activity (FRAP and DPPH method) and total carotenoid content. Fresh samples were extracted for all quality estimation.

Estimation of ascorbic acid

Ascorbic acid estimation was done at the marketable green fruit stage by the 2,6-dichlorophenol-indophenol titration method (Harris and Olliver, 1942). A sample weighing 5 g was extracted in 4% oxalic acid, and the volume was made up to 100 ml after centrifugation. 5 ml of working standard solution was pipetted into a 100 ml conical flask to which 10 ml of 4% oxalic acid was added and titrated against the dye (V_1 ml). 5 ml of supernatant was pipetted from the sample extracted, and 10 ml of 4% oxalic acid was added and titrated against the dye (V_2 ml). The ascorbic acid (mg/100g) was calculated as:

$$\text{Ascorbic acid (mg/100g)} = \frac{0.5\text{mg}}{V_1\text{ ml}} \times \frac{V_2\text{ ml}}{5\text{ ml}} \times \frac{100\text{ ml}}{\text{Wt. of the sample}} \times 100$$

Estimation of total phenols

Total phenols were estimated as per the procedure given by Singleton and Rossi (1965). Diluted sample 0.8 ml was taken in a test tube to which 7.7 ml of distilled water and 0.4 ml of Folin-Ciocalteau reagent was added, and were kept in B.O.D. at 40°C for 10 minutes. 1 ml of 20% sodium carbonate solution was added to all the test tubes and kept in the dark for incubation for one hour. The blue colour developed was read in a spectrophoto-meter at 660 nm.

Estimation of antioxidant contents

2.3.1. FRAP (Ferric reducing antioxidant power) method: 0.1 ml of the sample was taken into test tubes. 160 ml acetate + 16 ml $FeCl_3$ + 16 ml TPTZ was mixed, and the solution was prepared and poured them at 6ml to each test tube, including a blank containing 0.1 ml 80% alcohol. Then incubated for 1 hour, and readings were taken in a spectrophotometer at 593 nm.

2.3.2. DPPH (2,2-diphenyl-1-picrylhydrazyl) method:200 ml of DPPH was prepared by adding 8 mg DPPH to 200 ml of methanol. 6 ml of DPPH solution was added to test tubes containing 0.1 ml of the sample and incubated for an hour, and readings were taken at 517 nm using methanol as a blank.

Estimation of total carotenoid

3 g of the sample was extracted using acetone, and the volume was made up to 50 ml. Supernatant was taken from the sample, and readings were taken at wavelengths 450 nm, 470 nm, 644.8 nm, 661.6 and 503 nm in a spectrophotometer. Total carotenoids were measured as mg/100g fresh weight.

Statistical analysis

The method of analysis for combining ability estimation was carried out using Griffing (1956), and for estimation of heterosis in terms of increase or decrease in performance, measured as the proportion of deviation of F_1 from better parent and standard check (Indra) and expressed in percentage. All the statistical analysis was carried out using Indostat software.

I) Better parent heterosis (BPH): F1-BP/BP x 100
ii) Standard parent heterosis (SPH): F1- SP/SP x 100

Results and Discussion

Mean performance of hybrids with their parental lines

The mean performance of all parents and their F_1 hybrids revealed that the parents in this study were genetically different and had potential breeding values. The mean performance of 21 F_1 hybrids with their parental lines and commercial check hybrid is presented in Table 1. Among the parental genotypes,

Table 1: Mean performance of crosses and parents (FRAP, DPPH, total phenols antioxidant activity, ascorbic acid and carotenoid)

Sl. No.	Genotypes	Ascorbic acid	Carotenoids	Total phenols	FRAP	DPPH
Parents						
1.	AM	135.59	1.59	100	70.07	50.88
2.	AG	153.67	2.36	128.37	146.09	123.55
3.	AB	85.88	0.51	121.12	130.74	133.64
4.	YW	140.11	2.09	121.62	82.81	73.20
5.	CW	131.07	2.31	117.87	103.10	94.19
6.	UHFBP4	126.55	2.20	125.87	101.89	91.76
7.	CW308	140.11	1.46	131.12	106.07	91.76
Crosses						
8.	AM x AG	180.94	2.29	119.12	183.66	161.69
9.	AM x AB	144.63	0.94	124.37	100.08	100.13
10.	AM x YW	167.23	2.14	110.62	115.61	109.69
11.	AM x CW	117.51	1.32	111.24	98.50	90.43
12.	AM x UHFBP4	167.23	1.29	106.87	75.95	66.005
13.	AM x CW308	122.03	0.95	115.62	136.38	125.22
14.	AG x AB	171.75	1.53	152.50	183.99	157.16
15.	AG x YW	149.15	1.68	111.87	113.34	112.80
16.	AG x CW	203.29	1.66	139.37	94.12	157.92
17.	AG x UHFBP4	162.71	2.25	142.50	126.37	121.06
18.	AG x Cw308	216.95	1.52	107.47	108.33	71.25
19.	AB x YW	171.75	0.88	116.75	149.84	156.12
20.	AB x CW	198.87	1.06	161.04	94.12	89.99
21.	AB x UHFBP4	189.83	0.59	118.12	104.14	94.35
22.	AB x CW308	144.63	1.45	127.50	156.60	152.36
23.	YW x CW	149.15	3.07	119.37	111.32	102.89
24.	YW x UHFBP4	140.11	1.62	125.21	98.28	157.80
25.	YW x CW308	135.58	1.59	120.00	124.03	127.32
26.	CW x UHFBP4	126.55	1.57	130.21	97.06	105.26
27.	CW x CW308	162.71	1.03	117.71	106.11	111.27
28.	UHFBP4 x CW308	167.33	1.95	120.00	115.49	94.45

Commercial check						
29.	Indra	153.67	2.09	109.3	107.6	99.695
	SEm±	4.95	0.06	2.6898	2.5506	4.8676
	CD @ 5%	14.31	0.18	7.7799	7.3774	14.0789
	CD@ 1%	19.29	0.25	10.485	9.9426	18.9742

AM: Arka Mohini, AG: Arka Gaurav, AB: Arka Basant, YW: Yolo Wonder, CW: California Wonder.

the mean value ranged from 85.88 mg/100g (AB) to 153.67 mg/100g (AG) for ascorbic acid content, whereas it ranged from 117.51mg/100g (AM x CW) to 216.95 mg/100g (AG x CW308) in hybrids. On the other hand, carotenoid content varied from 0.51 (AB) mg/100g to 2.36 (AG) mg/100g in parents and 0.95 mg/100g (AM x CW308) to 3.07 mg/100g (YW x CW) in hybrids. Total phenol content ranged from 100 mg/100g (AM) to 131.12 mg/100g (CW308) in parents, and it varied from 106.87 mg/100g (AM x UHFBP4) to 161.04 mg/100g (AB x CW) in hybrids. Range of FRAP activity among parents was 70.07 mg/100g (AM) to 146.09 mg/100g (AG), whereas it ranged from 94.12 mg/100g (AG x CW). For DPPH antioxidant activity, values ranged from 50.88 mg/100g (AM) to 133.64 mg/100g (AB) among parents and 71.05 mg/ 100g (AG x CW308) to 161.69 mg/100g (AM x AG) among hybrids.

Analysis of general combining ability among parents and specific combining ability among crosses

A parent is said to be a good general combiner if it has a significant GCA effect. In this experiment, the parents and crosses were scored based on their GCA and SCA status. Significantly negative GCA and SCA were scored as "-1" and non-significant GCA and SCA were scored as "0", whereas "+1" score was given to significantly positive GCA and SCA effects. By taking these scores into consideration, parents and hybrids were classified as poor, average and good combiners (Supplementary Table 1, 2). For ascorbic acid content, AG (18.08) had a significant positive GCA effect. UHFBP-4 (0.34), AM (0.18) and YW (0.11) exhibited significant GCA effect for carotenoid content. For FRAP antioxidant activity, three parents had exhibited significant positive GCA effects, which were highest in AG (19.77) followed by AB (14.03). For DPPH antioxidant activity, AB (13.86) exhibited highest significant positive GCA effects followed by AG (15.15). The highest significant GCA was exhibited by AB (6.52), followed by AG (5.08), for total phenols (Table 2). These results suggest that, out of seven parents, Arka Gaurav and Arka Basant were considered to be good general combiners and can be used as the best source to improve the quality traits in bell pepper. With respect to SCA effects AM x CW308, AB x YW, AG x AB, BP4 x CW308 exhibited good SCA effects for most of the quality traits studied (Table 3). Out of 21 crosses, ten

Table 2: General combining ability (GCA) effects of parents for quality traits

Parent	Ascorbic acid	Carotenoids	FRAP	DPPH	Total phenols
Arka Mohini	-6.52 **	0.18 **	-8.19 **	-15.29 **	-10.66 **
Arka Gaurav	18.08**	-0.02	19.77 **	15.15 **	5.08**
Arka Basant	-4.02 *	-0.45 **	14.03 **	13.86 **	6.52 **
Yolo Wonder	-4.02 *	0.11 **	-5.11 **	2.27	-4.09 **
California Wonder	-1.02	0.04	-12.96 **	-5.16*	3.43 **
UHF-BP-4	-2.50	0.34 **	-11.44 **	-7.79**	1.20
CW308	0.005	-0.21 **	3.89 **	-3.03	-1.48
SEm±	1.48	0.03	0.78	1.51	0.83
CD at 5%	3.61	0.07	1.92	3.68	2.03
CD at 1%	5.47	0.11	2.90	5.58	3.08

Table 3: Specific combining ability (SCA) effects of crosses for quality traits

Crosses	Ascorbic acid	Carotenoids	FRAP	DPPH	Total phenols
AM x AG	15.70 **	0.03	56.57 **	50.26 **	1.73
AM x AB	1.49	0.56 **	-21.26 **	-10.02 *	5.54 *
AM x YW	24.09 **	-0.25 *	13.41 **	11.14 **	2.39
AM x CW	-28.63 **	0.69**	4.14*	-0.68	4.49*

AM x UHFBP4	22.58 **	0.15*	-19.93 **	-22.48 **	-6.64 *
AM x CW308	-25.13 **	0.57 **	25.17 **	31.97 **	4.79*
AG x AB	4.01	-0.09	34.68 **	16.57 **	17.91 **
AG x YW	-18.58**	-0.51 **	-16.82 **	-16.19 **	-12.10 **
AG x CW	32.55 **	-0.46 **	-28.19 **	36.36 **	7.88 **
AG x UHFBP4	-6.54	-0.16*	2.53	2.13	13.23 **
AG x CW308	45.18 **	0.58 **	-30.84 **	-52.46 **	-19.11 **
AB x YW	26.12 **	0.89 **	25.42 **	28.46 **	-8.67 **
AB x CW	50.24 **	-0.62 **	-22.45 **	-30.29 **	28.11 **
AB x UHFBP4	42.68 **	0.58 **	-13.95 **	-23.29 **	-12.58 **
AB x CW308	-5.03	0.03	23.17 **	29.95 **	-0.52
YW x CW	0.518	0.82 **	13.89 **	-5.79	-2.95
YW x UHFBP4	-7.038	-0.09	-0.67	51.75 **	5.11 *
YW x CW308	-14.08**	-0.40 **	9.74 **	16.50 **	2.58
CW x UHFBP4	-23.59**	0.17	5.95 *	6.64	2.59
CW x CW308	10.05 *	-0.89 **	-0.34	7.88*	-7.22**
UHFBP4 x CW308	16.153 **	0.51 **	7.53 **	-6.30	-2.71
SEm±	3.65	0.07	1.94	3.73	2.05
CD at 5%	7.62	0.15	4.04	7.77	4.29
CD at 1%	10.39	0.21	5.51	10.60	5.85

*and**: Significance at p= 0.05 and p= 0.01, respectively. AM: Arka Mohini, AG: Arka Gaurav, AB: Arka Basant, YW: Yolo Wonder, CW: California Wonder.

crosses were good specific combiners for ascorbic acid content and the maximum significant positive SCA effect was exhibited by the cross AB x CW (50.54), followed by AG x CW308 (45.18). Likewise, eight crosses exhibited positive significant SCA effects, out of which the highest significant SCA effect was shown by cross AB x YW (0.89) followed by YW x CW (0.82) for total carotenoid. Similarly, AM x AG (56.57) followed by AG x AB (34.68) for FRAP antioxidant activity. For DPPH antioxidant activity ten crosses had exhibited positive significant SCA effects and the highest was exhibited by the cross YW x UHFBP4 (51.75) followed by AM x AG (50.26). likewise for total phenols eight crosses exhibited significant positive SCA effects, and the cross AB x CW (28.11) followed by AG x AB (17.91) had the highest SCA effects. For all the quality traits estimated, it was found that the traits are governed by non-additive genes, hence, it is highly amenable for exploitation through heterosis. Similarly, Khalil and Hatem (2014), Hegde (2016), Praveen *et al*., (2017) and Aditika (2018) have reported the same for ascorbic acid content. The obtained results are supported by the studies of Navazio and Simon (2001) in cucumber, Tamilselvi *et al.,* (2015) in pumpkin for total carotenoids.

Estimation of the magnitude of heterosis for quality traits

By observing the mean values of parents and hybrids, it can be inferred that hybrids have performed good for the quality traits studied. Significant positive heterosis is desirable for all the quality traits studied. The better parent heterosis percentage ranged from 16.13 to 51.73, of which cross AB x CW (51.73) showed the highest heterobeltiosis followed by AB x UHFBP4 (50.00) for ascorbic acid content. Similarly, commercial heterosis percentage ranged from -17.64 to 41.18, of which the cross AG x CW308 (41.18) recorded the highest, followed by AG x CW (32.29) (Table 4). The results are similar in comparison with the studies by Sharma *et al*., (2013), Hegde (2016), Praveen *et al*., (2017) and Aditika (2018) in capsicum, where they have reported positive heterosis for ascorbic acid content. For carotenoid content, better parent heterosis ranged from -55.31 to 67.08, the cross AM x CW308 (67.08) showed the highest positive heterobeltiosis, followed by AM x AB (50.67) and commercial heterosis ranged from -50.49 to 46.76, of which the highest was reported by the cross YW x CW (46.76) followed by AM x CW (44.27) (Table 4). Similar results were obtained by Bhutia *et al*., (2015) in chilli and Roy *et al.,* (2018) in capsicum. Significant differences among genotypes for total phenols were observed, ranging from 106.87 (AM x UHFBP4) to 152.50 (AG x AB) among crosses. The heterobeltiosis ranged from -15.09 to 32.95 and the cross AB x CW (32.95) showed highest significant positive heterobeltiosis followed by AG x AB (18.79). Commercial heterosis ranged from -8.07 to 47.34 of which the highest positive commercial heterosis was

Table 4: Better parent and commercial heterosis for quality traits

Crosses	Ascorbic acid		Carotenoids		Total phenols		FRAP		DPPH	
	BP	CC	BP	CC	BP	CC	BP	CC	BP	CC
AM x AG	17.75 *	17.75 *	-3.19**	9.42**	-7.21	8.99 *	25.72 **	70.69 **	30.87 **	62.19 **
AM x AB	6.67	-5.88	50.67 **	13.67 **	2.68	13.79 **	-23.45 **	-6.98	-25.08 **	0.44
AM x YW	19.36 **	8.82	2.51**	2.51**	-9.04 *	1.21	39.61 **	7.44 *	49.85 **	10.03
AM x CW	-13.33	-23.53 **	30.23 **	44.27 **	-5.62	1.78	-4.46	-8.46 *	-3.99	-9.29
AM x UHFBP4	23.34 **	8.82	25.68 **	32.23 **	-15.09 **	-2.22	-25.46 **	-29.41 **	-28.07 **	-33.79 **
AM x CW308	-12.90	-20.59 **	67.08 **	26.06 **	-11.82 **	5.79	28.57 **	26.75 **	36.47 **	25.61 **
AG x AB	11.77 *	11.77	-35.38 **	-26.97 **	18.79 **	39.52 **	25.95 **	71.00 **	17.60**	57.65 **
AG x YW	-2.94	-2.94	-28.98 **	-19.72 **	-12.85 **	2.36	-22.41 **	5.34	-8.7	13.15
AG x CW	32.29 **	32.29 **	-29.80 **	-20.66 **	8.57 *	27.52 **	-35.57 **	-12.52 **	27.82 **	58.41 **
AG x UHFBP4	5.89	5.89	-4.61**	7.82**	11.00 **	30.38 **	-13.50 **	17.44 **	-2.01	21.44 **
AG x CW308	41.18**	41.18**	3.72**	17.24**	-18.04**	-1.67	-25.84**	0.68	-42.33**	-28.53**
AB x YW	22.58**	11.77	26.39**	26.39**	-4.01	6.82	14.60**	39.26**	16.82*	56.60**
AB x CW	51.73**	29.41**	-54.06**	-49.10**	32.95**	47.34**	-28.01**	-12.52**	-32.66**	-9.73
AB x UHFBP4	50.00**	23.53**	16.45**	22.52**	-6.16	8.07*	-20.34**	-3.21	-29.40**	-5.36
AB x CW308	3.23	-5.88	-0.51**	-30.43**	-2.76	16.65**	19.78**	45.54**	14.01	52.83**
YW x CW	6.45	-2.94	32.48**	46.76**	-1.85	9.22*	7.98*	3.46	9.23	3.2
YW x UHFBP4	0	-8.82	11.34**	17.14**	-0.53	14.56**	-3.55	-8.66*	71.97**	58.28**
YW x CW308	-3.23	-11.77	-24.00**	-24.00**	-8.48*	9.79*	16.93**	15.27**	38.75**	27.71**
CW x UHFBP4	-3.44	-17.64*	14.42**	26.75**	3.44	19.13**	-5.86	-9.80*	11.75	5.59
CW x CW308	16.13*	5.88	-55.31**	-50.49**	-10.23*	7.69	0.03	-1.38	18.13*	11.62
UHFBP4 x CW308	19.43**	8.89	23.95**	30.41**	-8.48*	9.79*	8.88*	7.33	2.93	-5.26
Sem±	4.95	4.95	0.06	0.06	2.69	2.69	2.55	2.55	4.87	4.87
CD@ 5%	14.31	14.31	0.18	0.18	7.78	7.78	7.38	7.38	14.08	14.08
CD@ 1%	19.29	19.29	0.25	0.25	10.49	10.49	9.94	9.94	18.97	18.97

*and**: Significance at P= 0.05, P= 0.01 respectively. BP: Heterosis percent over better parent. CC: heterosis over commercial check (Indra). AM: Arka Mohini, AG: Arka Gaurav, AB: Arka Basant, YW: Yolo Wonder, CW: California Wonder.

observed in AB x CW (47.34) followed by AG x AB (39.52) (Table 4). Positive heterosis for total phenols was also reported by Patel *et al*., (2017) in brinjal. For FRAP antioxidant activity, the heterobeltiosis ranged from -35.57 to 39.61and AM x CW308 (28.57) showed highest positive heterobeltiosis followed by AG x AB (25.95). Commercial heterosis ranged from -29.41 to 71.00 and highest commercial heterosis for this trait was observed in AG x AB (71.00) followed by AM x AG (70.69) (Table 4). The range of heterobeltiosis was -42.33 to 71.97 for DPPH activity. The cross YW UHFBP4 (71.97) showed highest significant positive heterobeltiosis, followed by AM x YW (49.85). Commercial heterosis for the same ranged from -33.79 to 62.19 and highest was observed in AM x AG (62.19) followed by AG x CW (58.41) (Table 4).

General and specific combining ability variances

General combining ability (GCA) variance, variance of specific combining ability (SCA) and GCA/SCA ratio are furnished in Table 5. All the studied traits showed a

Table 5: General and specific combining ability variance for quality parameters

Character	GCA	SCA	GCA: SCA
Ascorbic acid content	65.64	860.28	0.08
Carotenoids	0.07	0.34	0.20
Total phenols	34.89	130.31	0.27
DPPH antioxidant activity	123.62	860.04	0.14
FRAP antioxidant activity	165.09	590.72	0.28

GCA: General combining ability variance; SCA: Specific combining ability variance.

higher magnitude of SCA variance. The relatively lower GCA/SCA ratio for all the traits showed the preponderance of non-additive gene effects. Khalil and Hatem (2014), Tamilselvi *et al.,* (2015) in pumpkin, Hegde (2016), Praveen *et al*., (2017) and Aditika (2018) have reported the same.

Conclusion

The overall objective of this study was to improve the nutritional traits of F_1 hybrids in bell pepper developed through a half-diallel mating design. From the above results, the promising hybrids based on combining ability and heterosis for quality traits were Arka Gaurav x CW308, Arka Basant x California Wonder for ascorbic acid, Yolo Wonder x California Wonder for carotenoid, Yolo Wonder x UHFBP4 and Arka Mohini x Arka Gaurav for DPPH activity, Arka Gaurav x Arka Basant and Arka Mohini x Arka Gaurav for FRAP, Arka Basant x California Wonder and Arka Gaurav x Arka Basant for total phenols. Thus, the best performing crosses have potential to be exploited. The present study has also inferred a preponderance of non-additive gene action for quality traits where heterosis breeding could be more useful for improvement of quality traits in bell pepper.

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RESEARCH ARTICLE

Studies on Genetic Divergence in Tulsi (*Ocimum basilicum*) under Semi-Arid Conditions for the Development of Novel Cultivars

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Abstract

In the present investigation, 40 Tulsi (*Ocimum basilicum*) genotypes were grown in RBD in the Research Area of Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. The observations were recorded for 17 quantitative traits. All the genotypes were grouped into 10 different clusters, demonstrating the presence of genetic divergence among the genotypes studied. The maximum intra-cluster distance was recorded for cluster VII and maximum inter-cluster distance was recorded between cluster II and cluster X. Relative contribution of diverse traits towards genetic diversity analysis revealed that 1000 seed weight contributed maximum (41.41%) towards genetic divergence followed by number of spikes per plant (17.31%), fresh herbage yield per plant (13.46%) and oil content (10.77%). Based on the maximum mean performance for seed yield, number of primary branches, fresh as well as dry herbage yield, oil content and seed vigour index-I & II, the genotypes EC 338772, EC 388890 and IC 326732 may be exploited in the breeding program for the development of novel improved cultivars of Tulsi for higher oil content, seed yield, and other economic traits.

Keywords: Genetic divergence, *Ocimum spp*., Semi-arid conditions, Tulsi

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Introduction

Tulsi is a medicinal and aromatic crop plant that belongs to the Lamiaceae family and the *Ocimum* genus. In India, nine species of *Ocimum* (*Ocimum tenuiflorum* L., *O. basilicum* L., *O. gratissimum* L., *O. kilimandscharicum* L., *O. micranthum* L., *O. campechianum* L., *O. americanum* L., *O. minimum* L. and *O. citriodorum* L.) have been reported of which the last three are exotic species (Balyan & Pushpangadan, 1988; Talawade *et al*., 2024). The *Ocimum basilicum* and *O. sanctum* species are most widely distributed in India (Arya *et al*., 2021). *Ocimum americanum* and *O. canum* are two species that are primarily found in north-western India, including Jammu and Kashmir, Punjab, Himachal Pradesh, Delhi and Uttar Pradesh. *O. gratissimum* is found across North India (Krishnamoorthy, 1989). The *O. basilicum* is known by various names such as 'Sweet Basil', 'Common Basil' or 'French Basil'. The *O. canum* species with a peculiar mint smell is known as 'Mint Basil'. The camphor-containing species *O. kilimandscharicum* is commonly called 'Camphor Basil'. The species *O. canum,* which has a borneol smell, is known as 'Hoasy Basil', and the species *O.*

gratissimum with high contents of eugenol is known as 'Spice Basil'. Hindus worship the plants of *O. sanctum,* hence it is popularly known as 'Sacred Basil' or 'Holy Basil' (Talawade *et al*., 2024). The genus *Ocimum* exhibits a range of chromosome numbers, including various haploid chromosome numbers (12, 13, 16, 20, 24, 32, 36 and 38) in addition to the basic chromosome number. According to Carovic *et al*. (2010), the basic chromosome number for *Ocimum species* is x=12. Moreover, *O. basilicum* and *O. americanum* are known to be tetraploid (2n=4x=48) and hexaploid (2n=6x=72), respectively (Sobti and Pushpangadan, 1979).

Genetic diversity plays an important role in plant breeding, either to generate productive recombinants or to exploit heterosis (Chowdhury *et al*., 2017). The divergence analysis can be a valuable instrument in measuring the extent of divergence between distinct germplasm lines (Bompalli and Nallabilli, 2013). Thus, a detailed understanding of the type and extent of genetic divergence can aid plant breeders in identifying genetically diverse plants that can be used in crop improvement programmes for the development of new recombinants (Arunachalam, 1981).

Although *Ocimum* possesses medicinal and aromatic properties of high value, it is not commonly grown on a large scale. It may become a significant crop in the near future as a result of the increasing demand for aromatic compounds in the food, flavours and pharmaceutical industries. The low cultivation area and yield of *Ocimum* can be attributed to the unavailability of genotypes that are suitable for the specific regions. When selecting *Tulsi* for cultivation*,* farmers consider the yield and quality of the herb and oil, as well as other related characteristics that contribute to an increased economic yield and profit. It is essential to conduct studies of different genotypes across various agro-climatic regions to identify the best-yielding genotypes with unique attributes that influence the yield. This approach will allow us to recognize promising genotypes that can be used in breeding programmes to improve the yield of sacred basil. Therefore, keeping the above points in view, the present investigation has been planned to determine the extent of genetic diversity for different traits to classify the *Tulsi* genotypes into different clusters.

Materials and Methods

Experimental Material

The experimental material for the present investigation was comprised of 40 genotypes of Tulsi (*Ocimum basilicum*) (IC44681, EC388887, IC387837, EC388895, IC369247, EC388896, IC387838, EC388782, IC388785, EC388737, IC469938, EC388889, IC326735, EC338772, IC312264, EC388788, IC110207, EC388890, IC338794, IC436153, IC336833, IC381158, IC201223, IC326732, IC328582, RDV 45, IC338959, NSV38, IC333833, EC112548, IC281185, IC75730, IC381552, Local 1, EC469904, IC381185, EC326771, Local 2, EC388893, and DOS 1). Among these, 38 genotypes were received from ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi and 2 genotypes from the germplasm pool maintained at the Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar.

Field layout of the Experiment

To conduct the field experiment, all 40 genotypes of Tulsi (*Ocimum basilicum*) were planted in the Research Field of the Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, during the 2022 and 2023 *kharif* seasons. Hisar is located in a semi-arid sub-tropical region at 29.10°N latitude and 75.46°E longitude with an elevation of 215.52 m above mean sea level.

Nursery Preparation and Transplanting

The nursery was raised by sowing the seeds of all 40 genotypes on separate raised beds. Light irrigation was given from sowing to the development of seedlings with good growth. Twenty-day-old young healthy seedlings of all the genotypes were transplanted in the Randomized Block Design with three replications. One seedling per hill was transplanted at 10 cm spacing between the plants in two rows, each of 3m length, with a row-to-row distance of 30 cm. A light irrigation was applied immediately after transplanting. All the required cultural practices were followed to raise a good *Tulsi* crop.

Observations and Analysis

The observations for following 17 quantitative traits i.e. plant height (cm), number of primary branches/plant, days to 50% flowering, spike length (cm), number of spikes/plant, number of flowers' whorls/spike, fresh herbage yield/plant (g), oil content (%), dry herbage yield/plant (g), days to maturity, seed yield/plant (g), 1000 seed weight (g), standard germination (%), seedling dry weight (mg), seedling length (mm), seed vigour index – I, and seed vigour index – II were recorded under natural field conditions and in laboratory. The data for quantitative traits from 1 to 10 were recorded on five randomly selected competitive plants from each genotype in each replication of the

Tulsi crop grown under natural field conditions. Mean of the five plants was calculated to record the data on per-plant basis. The observations for the quantitative traits from 11 to 17 were recorded under laboratory conditions. The observations for 1000 seed weight (g), standard germination (%), seedling length (mm), seedling dry weight (mg), seed vigour index-I and seed vigour index-II were taken in Seed Testing Laboratory, Department of Seed Science and Technology, and for oil content (%) in the Quality Analysis Laboratory, Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. Analysis of variance (ANOVA) for the observations recorded on different characteristics was carried out as per the standard procedure suggested by Panse and Sukhatme (1967).

Results

Analysis of Variance (ANOVA)

The mean sum of squares due to genotypes presented in ANOVA Table 1, were highly significant for all the 17 quantitative traits *viz*., days to 50% flowering, days to maturity, number of primary branches per plant, plant height (cm), number of spikes per plant, spike length (cm), number of flowers' whorls per spike, fresh herbage yield per plant (g), dry herbage yield per plant (g), seed yield per plant (g), 1000-seed weight (g), oil content (%), standard germination (%), seedling length (cm), seedling dry weight (mg), seed vigour index-I and seed vigour index-II. It showed that an adequate amount of genetic variability was present for all the traits among the 40 genotypes studied, which can be utilized for genetic enhancement of Tulsi genotypes.

Genetic Divergence Analysis

In the present investigation, the D^2 distance matrix given by Mahalanobis (1936) and further elaborated by Murthy and Arunachalam (1966) was used for the genetic divergence study among the 40 *Tulsi* genotypes. The 40 *Tulsi* genotypes were grouped into 10 clusters by following Tocher's method (Rao 1952). The D^2 statistical analysis revealed that a substantial amount of genetic diversity is available among the 40 *Tulsi* genotypes. The genotypes that belong to the same cluster were more closely related to each other. A maximum of 29 genotypes were grouped in cluster I, followed by cluster VII (3 genotypes), and only one genotype was included under the cluster II, cluster III, cluster IV, cluster V, cluster VI, cluster VIII, cluster IX and cluster X as revealed in Table 2 and Fig. 1. Intra and inter-cluster distances are presented in Table 3. The maximum intra-cluster distance (35.57) was noticed for

Table 1: Analysis of variance (ANOVA) for seed yield and its component traits in 40 Tulsi genotypes

Sr. No.	Characters	Mean sum of squares		
		Replication	Genotype	Error
	Degree of Freedom	2	39	78
1.	Days to 50% flowering	4.01	504.77**	4.85
2.	Days to maturity	5.73	371.32**	4.96
3.	Number of primary branches/plants	1.70	19.35**	1.10
4.	Plant height (cm)	25.80	275.33**	11.85
5.	Number of spikes/plant	17.79	876.71**	21.03
6.	Spike length (cm)	0.31	37.54**	0.86
7.	Number of flowers' whorls/spike	0.06	11.58**	0.83
8.	Fresh herbage yield/plant (g)	486.91	163239.79**	804.67
9.	Dry herbage yield/plant (g)	385.20	21752.76**	130.25
10.	Seed yield/plant (g)	5.59	561.88**	7.58
11.	1000 seed weight (g)	0.002	0.482**	0.001
12.	Oil content (%)	0.0003	0.0222**	0.0001
13.	Standard germination (%)	12.10	453.80**	6.19
14.	Seedling length (mm)	1.58	692.94**	37.05
15.	Seedling dry weight (mg)	0.29	16.36**	0.11
16.	Seed vigour index-I	79664.43	8615014.11**	224980.60
17.	Seed vigour index-II	2124.72	119627.79**	1203.10

** Significant at 1% level of significance, *Significant at 5 % level of significance, DF: Degree of freedom

Table 2: Clustering of 40 Tulsi genotypes based on the D^2 statistic

Cluster	No. of genotypes	Name of genotypes
1	29	EC 388887, NSV 38, IC 281185, EC 388895, IC 333833, IC 336833, EC 388737, IC 436153, IC 326735, EC 388782, EC 388896, EC 112548, IC 326732, IC 338794, EC 388788, EC 388889, IC 312264, EC 326771, IC 381552, EC 469904, EC 388893, IC 381158, IC 338959, IC 387838, Local 1, IC 369247, IC 110207, EC 338772, IC 387837
2	1	IC 44681
3	1	IC 201223
4	1	IC 328582
5	1	RDV 45
6	1	IC 469938
7	3	IC 75730, Local 2, IC 381185
8	1	IC 388785
9	1	EC 388890
10	1	DOS 1

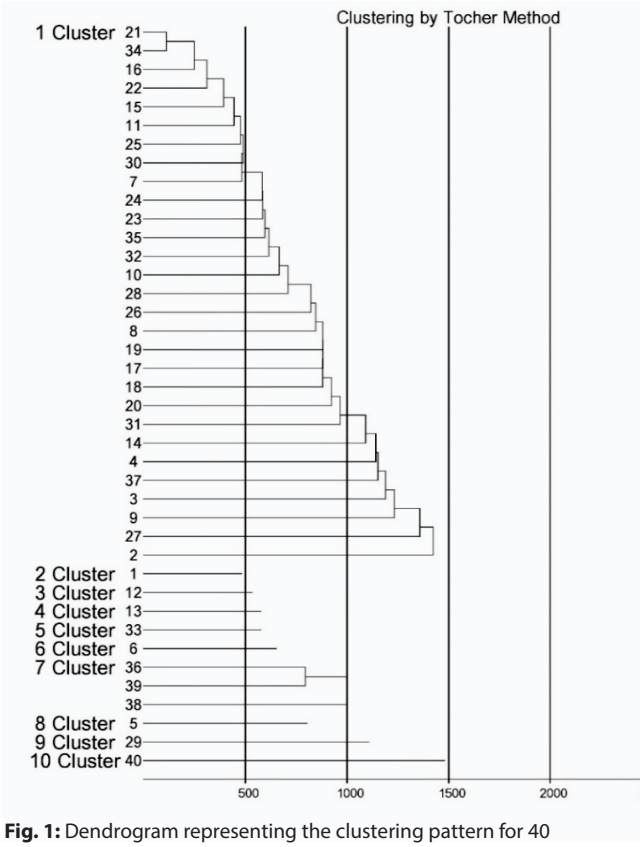

Fig. 1: Dendrogram representing the clustering pattern for 40 genotypes of Tulsi

cluster VII, followed by cluster I (31.73). For the rest of the clusters (cluster II, cluster III, cluster IV, cluster V, cluster VI, cluster VIII, cluster IX and cluster X) intra-cluster distance was zero. The maximum inter-cluster distance (105.80) was observed between cluster II and cluster X followed by between cluster IV and cluster X (99.80), cluster III and cluster X (98.65), cluster I and cluster X (83.26), cluster VIII and cluster X (83.08), cluster III and cluster VII (77.76), cluster II and cluster VII (77.11), cluster IV and cluster VII (76.30), cluster VI and cluster X (76.18), cluster V and cluster X (75.90), cluster V and cluster VII (68.68), cluster IX and cluster X (62.76) and cluster IV and cluster IX (59.63).

Cluster Means for Different Quantitative Traits of Tulsi Genotypes

The genetic divergence among 40 genotypes of *Tulsi* was also supported by cluster means computed for 17

Table 3: Intra and Inter Cluster distances (D^2) among ten clusters of Tulsi genotypes

Clusters	C 1	C 2	C 3	C 4	C 5	C 6	C 7	C 8	C 9	C 10
C 1	31.73	38.53	41.39	43.81	45.80	40.57	57.51	40.25	48.21	83.26
C 2		0.00	23.47	26.99	49.15	51.68	77.11	33.57	59.16	105.80
C 3			0.00	33.16	36.29	60.03	77.76	32.34	60.18	98.65
C 4				0.00	51.17	54.59	76.30	32.08	59.63	99.80
C 5					0.00	62.53	68.68	39.94	50.34	75.90
C 6						0.00	45.85	41.28	33.71	76.18
C 7							35.57	65.27	52.13	58.04
C 8								0.00	36.69	83.08
C 9									0.00	62.76
C 10										0.00

C 1: Cluster 1, C 2: Cluster 2, C 3: Cluster 3, C 4: Cluster 4, C 5: Cluster 5, C 6: Cluster 6, C 7: Cluster 7,C 8: Cluster 8, C 9: Cluster 9, C 10: Cluster 10

Table 4: Cluster means for 17 different quantitative traits of 40 Tulsi genotypes

Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X
DF	58.02	73.33	80.33	78.33	49.33	57.33	50.33	79.33	46.00	41.00
DM	165.67	168.00	166.67	160.00	160.33	159.33	153.89	156.67	168.13	140.00
PB	15.96	17.67	14.60	15.60	14.87	14.27	17.83	10.67	19.13	17.13
PH	83.96	88.70	104.60	104.03	85.97	84.90	93.77	103.13	87.13	81.00N
SP	101.92	66.00	120.00	89.80	232.03	81.00	121.12	146.60	195.23	326.40
SL	16.36	23.30	16.87	17.23	17.10	17.77	12.12	22.47	16.73	10.43
NFW	12.99	15.13	16.40	16.07	16.60	14.60	13.71	17.80	12.73	14.27
FHY	520.72	713.27	458.87	1003.43	382.50	1087.77	683.29	1026.33	1249.97	825.23
DHY	154.02	215.77	123.77	319.30	117.67	382.10	182.71	382.87	414.87	206.77
SY	27.76	33.73	20.80	48.70	60.90	16.13	13.52	38.17	57.07	30.47
SW	1.02	1.59	1.86	1.54	1.61	0.63	0.36	1.38	0.81	0.24
OC	0.21	0.26	0.21	0.15	0.22	0.40	0.31	0.33	0.49	0.24
SG	67.22	66.00	70.67	68.00	52.67	44.67	43.78	54.00	70.87	62.67
SDL	100.75	104.00	94.00	93.67	86.00	81.33	61.22	110.00	97.33	63.33S
DW	10.04	11.53	10.23	11.63	7.90	9.43	3.49	9.47	10.03	3.77
SVI-I	6781.65	6861.33	6644.00	6365.33	4537.33	3628.00	2694.22	5941.33	6878.00	3969.33
SVI-II	673.24	761.07	723.33	790.93	416.87	421.73	154.22	511.67	709.20	236.47

DF: Days to 50% flowering, DM: Days to maturity, PB: Number of primary branches/plant, PH: Plant height (cm), NSP: Number of spikes/plant, SL: Spike length (cm), FW: Number of flowers' whorls/spike, FHY: Fresh herbage yield/plant (g). DHY: Dry herbage yield/plant (g), SY: Seed yield/plant (g), SW: 1000 seed weight (g), OC: Oil content (%), SG: Standard germination (%), SDL: Seedling length (mm), SDW: Seedling dry weight (mg), SVI-I: Seed vigour index-I, SVI-II: Seed vigour index-II.

different quantitative traits (Table 4). The results revealed that cluster II showed the maximum cluster mean for spike length (23.30), cluster III for days to 50% flowering (80.33), plant height (104.60) and 1000-seed weight (1.86), cluster IV for seedling dry weight (11.63) and seed vigour index-II (790.93), cluster V for seed yield (60.90), cluster VIII for number of flowers' whorls per spike (17.80) and seedling length (110.00), cluster IX for days to maturity (168.13), number of primary branches per plant (19.13), fresh herbage yield per plant (1249.97), dry herbage yield per plant (414.87), oil content (0.49), standard germination (70.87) and seed vigour index-I (6878.00) and cluster 10 for number of spikes per plant (326.40). From this, it is clear that cluster IX exhibited the highest cluster means for the maximum (i.e. 7) number of characters. Whereas, the minimum cluster mean was shown by cluster II for number of spikes per plant (66.00), cluster IV for oil content (0.15), cluster V for fresh herbage yield per plant (382.50) and dry herbage yield per plant (117.67), cluster VII for seed yield per plant (13.52), standard germination (43.78), seedling length (61.22), seedling dry weight (3.49), seed vigour index-I (2694.22) and seed vigour index-II (154.22), cluster VIII for number of primary branches per plant (10.67), cluster XI for number of flowers' whorls per spike (12.73) and cluster X for days to 50% flowering (41.00), days to maturity (140.00), plant height (81.00), spike length (10.43) and 1000 seed weight (0.24).

Relative Contribution of Different Traits Towards Genetic Diversity

Based on the study of 40 genotypes of *Tulsi* regarding the relative contribution of the 17 yield and its attributes towards genetic divergence is presented in Table 5. It was observed that 1000 seed weight (41.41%)

Table 5: Relative contribution towards the genetic diversity of yield and its attributes

Trait	Contribution (%)
1000 seed weight (g)	41.41
Number of spikes/plant	17.31
Fresh herbage yield/plant (g)	13.46
Oil content (%)	10.77
Seedling dry weight (mg)	5.00
Standard germination (%)	3.59
Days to 50% flowering	2.69
Days to maturity	2.05
Seed vigour index-II	1.54
Dry herbage yield/plant (g)	1.15
Seed yield/plant (g)	0.64
Spike length (cm)	0.38

Trait	Contribution (%)
Plant height (cm)	0.00
Number of primary branches/plants	0.00
Seedling length (mm)	0.00
Number of flowers' whorls/spike	0.00
Seed vigour index-I	0.00

contributed maximum towards genetic divergence followed by number of spikes per plant (17.31%), fresh herbage yield per plant (13.46%), oil content (10.77%), seedling dry weight (5.00%), standard germination (3.59%), days to 50% flowering (2.69%), days to maturity (2.05%), seed vigour index-II (1.54%), dry herbage yield per plant (1.15%), seed yield per plant (0.64) and spike length (0.38%).

Discussion

Tulsi (*Ocimum spp.*) is one of the most important medicinal and aromatic crop plants that have been traditionally used in *Ayurvedic* medicine for its therapeutic properties (Saran *et al*., 2017). It is a versatile crop that can be grown by farmers with limited resources. The conservation and utilization of genetic resources by providing the genetically divergent germplasm lines to develop the improved genotypes (cultivars) for higher yield, disease resistance and high essential oil content, which are important for commercial cultivation (Patel *et al*., 2015; Koli *et al*., 2022). Yield is a complex trait that is influenced by various factors. In order to select the best genotypes for a particular environment, it is important to understand the relationship between yield and its attributing traits (Gowda *et al*., 2019). Wider adaptability and morphological stability an important criteria to improve the herbage yield, and quality of oil and economic products. Arya *et al*., (2024) reported a wide variation in adaptability and morphology in lemongrass. Although advances have been made in the breeding of some medicinal plants, several challenges remain, owing to the particularity and complexity in determining the breeding target. Additionally, in medicinal plants, there are limitations associated with research on traditional and modern breeding methods (Koli *et al*., 2021).

Genetic diversity analysis in plant breeding refers to the assessment of genetic variations within populations, which helps in selecting diverse parents for the improvement of traits and enhances the overall adaptability. Genetic diversity studies are also essential to ensure the resilience and adaptability of crop plants to changing environmental conditions, pests, and diseases. These studies help to identify and conserve diverse genetic resources, enabling breeders to develop new varieties with improved traits, higher productivity and enhanced resistance to biotic and abiotic stresses (Singh *et al*., 2010). Genetic divergence analysis (D^2 analysis) was done using the method suggested by Mahalanobis (1936) and elaborated by Murty and Arunachalam (1966). The level of genetic divergence between two genotypes increases with an increase in the range of D^2 values between them. The grouping of genotypes into different clusters was done following the Tochers' method as described by Rao (1952). A dendrogram was also plotted for genetic divergence studies of 40 *Tulsi* genotypes, which offered a visual representation that simplifies the understanding of genetic divergence. The D^2 analysis grouped the 40 *Tulsi* genotypes into 10 different clusters, which supports the existence of a substantial amount of genetic diversity among them.

After grouping the 40 genotypes of *Tulsi* into 10 different clusters based on 17 different quantitative traits, the mean values of the different clusters revealed the existence of adequate genetic variability among the 10 clusters in terms of the traits studied. In cluster IX, the highest cluster mean values were found for the large number of traits *viz*., seed vigour index-I (6878.00), fresh herbage yield per plant (1249.97), dry herbage yield per plant (414.87), days to maturity (168.13), standard germination (70.87), number of primary branches per plant (19.13) and oil content (0.49). Therefore, the genotype EC 388890 of cluster IX may be utilised comprehensively for further genetic improvement of the *Tulsi* crop. Relative contribution (%) of all 17 traits in the genetic diversity discovered that 1000 seed weight (41.41%) gave the maximum contribution in total genetic divergence expressed in the study. It was followed by the number of spikes per plant, with a 17.31% contribution, fresh herbage yield per plant, with a 13.46% contribution, and oil content, with a 10.77% contribution. The earlier research workers, Patel *et al*., (2018), Singh *et al.,* (2018), and Srivastava *et al.,* (2018) also reported similar findings regarding genetic diversity studies in *Ocimum spp*.

Conclusion

The present investigation on *Tulsi* genotypes revealed high genetic diversity among the genotypes. The maximum intra-cluster distance was observed for the cluster VII and cluster I whereas, the maximum inter-cluster distance was observed between cluster II and cluster X. The highest cluster means were observed in cluster IX for maximum number of characters *viz*. days

to maturity, number of primary branches per plant, fresh herbage yield per plant, dry herbage yield per plant, oil content, standard germination and seed vigour index-I. Relative contribution of different traits towards genetic diversity revealed that 1000 seed weight contributed the maximum towards genetic divergence, followed by number of spikes per plant, fresh herbage yield per plant and oil content. The maximum seed yield per plant and number of primary branches per plant were observed for the genotype EC 338772, maximum fresh as well as dry herbage yield per plant and maximum oil content were recorded for EC 388890, and seed vigour index I and II were found to be maximum for the genotype IC 326732. Hence, the genotypes EC 338772, EC 388890 and IC 326732 can further be utilised for the development of novel cultivars.

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RESEARCH ARTICLE

Morphological DUS Characterization and Genetic Diversity Analysis of Maize (*Zea mays* L.) Inbred Lines under Waterlogging Conditions

Tosh Garg[1], Sittal Thapa[1] and Rumesh Ranjan[1]*

Abstract

A comprehensive study was undertaken to characterize 80 maize (*Zea mays* L.) inbred lines for 28 morphological DUS traits under waterlogging (WL) conditions. The descriptors included 20 visually assessed and 8 measurable characteristics. Analysis of variance (ANOVA) for measurable traits revealed highly significant differences among genotypes for all characters studied, indicating the presence of substantial genetic variability. Visually assessed DUS traits showed wide variation among lines for kernel shape, glume colour, and grain colour, except for grain type, since all entries belonged to the field corn group. Cluster analysis based on combined visual and measurable traits grouped the genotypes into two major clusters, indicating clear phenotypic diversity. When clustering was performed using measurable traits alone, SE 610, SE 601, SE 616, I 190 and I 202 formed a separate cluster, highlighting their distinct morphological expression. The combined assessment of qualitative and quantitative DUS descriptors proved efficient in differentiating maize inbred lines. The study provides valuable baseline information for varietal distinctiveness testing, genetic purity maintenance, and selection of potential parents in breeding programs aimed at improving waterlogging tolerance in maize.

Keywords: Cluster analysis, Distinctness, Morphological traits, Stability, Uniformity, Waterlogging duration

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Introduction

Maize is an industrially important raw product for the production of myriad products such as starch, biofuel, beverages, oils and so on. Attributed to its highest genetic yield potential amongst all cereals, Maize is undoubtedly the Queen of cereals, being an important source of carbohydrates in the diet among developing countries and as a main feed to the livestock in developed countries. Endowed with a wide adaptability habit, this crop of maize is grown in almost every corner of the world, encompassing more than 165 countries and thriving in almost every edaphic and climatic condition. Globally, maize is an important cereal ranking second in the world in terms of acreage, cultivated on nearly 205 million hectares with global production of approximately 1.21 billion metric tonnes in 2023-24 (FAOSTAT 2025). Maize is an important crop for farmers in the northwestern plain (NWP) zone of India, providing both income and food for local communities. The majority of the area under the NWP zone is covered by paddy, and due to the decline in the groundwater table of this region, there is a call from policymakers to diversify the paddy with maize crops. In the northwestern plain zone of India, maize is typically grown during the *kharif* season, which coincides with

the rainy season and due to most of the lowland paddy area, waterlogging (WL) is a major problem for maize crops. WL is a condition where the soil is saturated with water for a prolonged period, which results in reduced oxygen availability to the plant roots, leading to several physiological and metabolic disorders (Rana *et al.,* 2025; Kumar *et al.,* 2025a). Maize is a water-loving crop and requires adequate moisture for growth and development. However, prolonged WL can have detrimental effects on maize growth and yield. WL in maize can lead to reduced plant growth and stunted roots, which ultimately result in decreased yield. It can also lead to an increase in disease incidence, such as root rot and fungal diseases, which can further affect the plant's growth and yield. Additionally, WL can also increase nutrient leaching and soil compaction, which can affect the plant's ability to uptake essential nutrients. To mitigate the effects of WL on maize, farmers can employ several strategies (Zaidi *et al.,* 2004; Kumar *et al.,* 2025 b). One of the best strategies is to select maize varieties that are tolerant to WL, or avoid planting maize in areas with poor drainage, which is hardly possible.

Screening is the first and foremost plant breeding activity, and to characterize the screened germplasm based on morphological features is mandatory. For characterization, the DUS (Distinctness Uniformity and Stability) test is carried out for all types of crops. DUS testing involves raising the crops under ambient growth conditions and collecting several morphological parameters to distinguish plants based on several visual and measurement assessments (Yadav and Singh, 2010). In India, the Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) has recognised 31 characters as the basic requirement for the characterization of maize genotypes for DUS (Anonymous 2007). Punjab Agricultural University, Ludhiana, is a reservoir of the maize germplasm of different maturity groups along with speciality maize. Maize breeding at PAU started during the 60's and developed several inbred lines with different utilities (Sandhu *et al.,* 2021). This developed germplasm is also classified into different heterotic pools and is being used by different centres in their breeding program. The objective of this study was to characterise the set of inbred lines tested for WL tolerance at different durations morphologically.

Materials and methods

A set of eighty different genotypes of different heterotic pools that were also tested for WL tolerance at different durations, *viz.,* 3, 6 and 9 days of flooding, were sown in the experiment area of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India, in the year 2020-21. All recommended packages of practices were followed. The experimental materials procured for DUS testing are listed in Table S1:

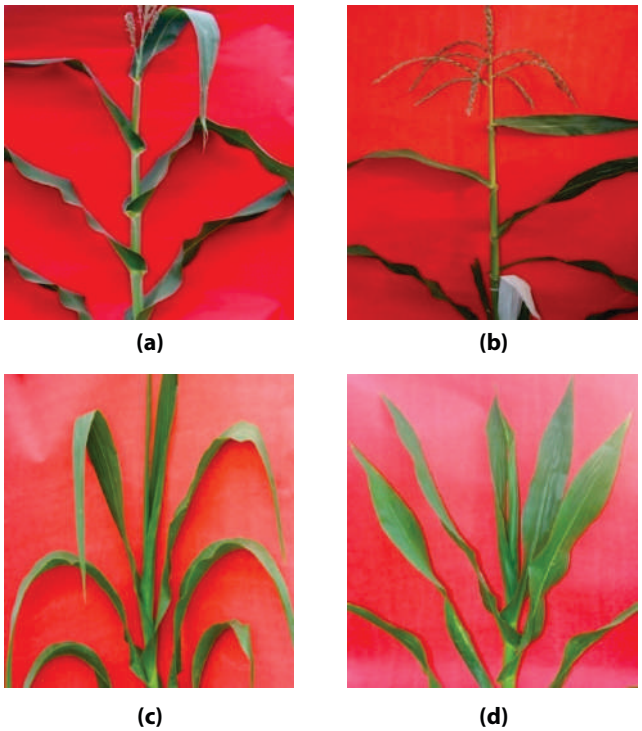
The experiment was conducted in Randomised Block Design (RBD) with three replications, having 2 rows per genotype with row length of 4 m. The plant-to-plant spacing was 20 cm, and row-to-row spacing was 60 cm. Observations for a genotype were made from 18 plants (6 plants selected randomly from each replication) following the guidelines as per PPV&FR authority (Anonymous 2007).

Observations were recorded at different growth stages and taken from the different plant parts. Four types of assessments were followed:

1. **VS:** Visual assessment by observations of individual plants or parts of plants
2. **VG:** Visual assessment by a single observation of a group of plants or parts of plants
3. **MS:** Measurement of a number of individual plants or parts of plants
4. **MG:** Measurement by a single observation of a group of plants or parts of plants

For visually assessed characteristics, no statistical methods were employed, and their expression level was used for assessment of their distinctness.

A total of 28 morphological characteristics (Fig. 1) were taken into account to distinguish 80 maize inbred



(a) **(b)**

(c) **(d)**

(e)

(f)

(m)

(n)

(g)

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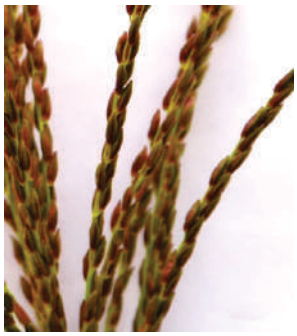
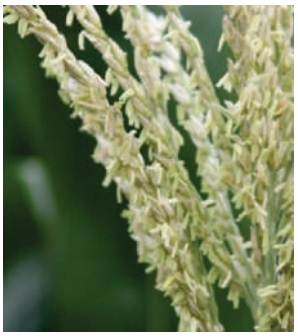


(i)



(j)

(q)

(r)

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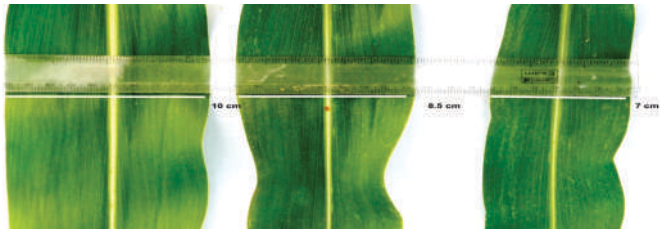


(ac)

Fig. 1: Different visually assessed DUS characters observed at field condition.

Angle between stem and leaf blade a: acute, b: wide; Attitude of leaf blade c: droopy, d: straight; anthocyanin pigment e: present, f: absent; anthocyanin at base of glume g: present, h: absent; anthocyanin colouration of glumes i: present, j: absent; anthocyanin colouration of anthers k: present, l: absent; spikelet density m: dense, n: sparse; tassel angle o: normal, p: wide; anthesis of tassel q: straight, r: curved; anthocyanin colouration of silk s: present, t: absent; ear placement u: low and high, v: medium, tassel length w: short, x: medium, y: long; plant height z: short, aa: medium, ab: long; ac: different leaf width.

lines, which included 20 visually assessed characteristics and 8 measurable characteristics. Twenty visually assessed and eight measurable DUS traits were recorded in field conditions at their respective crop growth period. Traits including plant height (cm), width of leaf blade (cm), anthesis period (days), length of tassel (cm), silking period (days), cob length (cm), cob diameter (cm) and grain weight (gm) were eight measurable DUS characters. A Vernier Calliper was used for taking cob length and cob diameter, while a long (1-meter) scale was used for plant height and leaf blade width data recording. 1000 grain weight was measured using a standard weighing balance.

Statistical analysis

The lines subjected to DUS characterization were sown in three replications in a Randomized Block Design (RBD). All the measurable characteristics were recorded from six plants for each line and each replication. An average reading was calculated and subjected to one-factor analysis. Significant differences were observed among the inbred lines for all the measurable characters. ANOVA for eight measurable DUS characters and clustering was calculated using the R software package.

Results and Discussion

A total of 28 morphological characteristics were taken into account to distinguish 80 maize inbred lines, which included 20 visually assessed characteristics and 8 measurable characteristics. Data collected during the experiment were analysed, and the results obtained were presented under different sections.

Table 1: Analysis of Variance (ANOVA) statistics for measurable DUS characters

Source of variation	df	Mean squares							
		Cob length	Cob Diameter	Tassel length	Plant height	Silking Days	Leaf blade width	Anthesis days	Kernel 1000 grains
Replication	2	5.064*	0.255^{NS}	0.487^{NS}	75.8165*	2.037^{NS}	0.075^{NS}	2.2625^{NS}	16.559*
Treatment	79	11.95*	0.457*	106.93*	1285.53*	48.881*	4.201*	47.615*	8491.393*
Error	239	1.177	0.072	0.905	17.677	2.379	0.026	2.895	112.560

* = significant at 5% probability level

Measurable DUS Characteristics

The analysis of variance (ANOVA) for measurable DUS characters revealed highly significant differences among treatments for all the traits studied (Table 1). This indicates the presence of substantial genetic variability among the genotypes evaluated under WL conditions. Significant mean squares due to treatments were observed for cob length, cob diameter, tassel length, plant height, days to silking, leaf blade width, days to anthesis, and kernel weight of 1000 grains. The significant variability among genotypes for morphological traits reflects differential genotypic responses to WL stress.

Variability in plant height and tassel length under excess moisture stress indicates differential tolerance mechanisms influencing vegetative growth and reproductive organ development. A marked reduction in plant stature under prolonged WL stress has been reported earlier due to restricted root aeration and impaired nutrient uptake (Zaidi *et al.,* 2010).

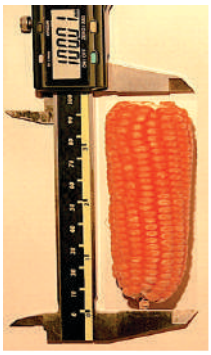
The significant treatment effects for anthesis and silking days suggest that genotypes responded differently in terms of phenological adaptation under stress. A delay in anthesis and silking is often observed under hypoxic conditions as a result of disrupted hormonal signalling and carbohydrate transport (Setter and Waters, 2003; Baishan *et al.,* 2010).

The significant differences in leaf blade width and cob diameter also indicate that genotypes vary in their ability to maintain photosynthetic surface area and ear development under waterlogged environments. Such diversity among genotypes is desirable for the selection of potential parents in breeding programs aimed at improving WL tolerance in maize.

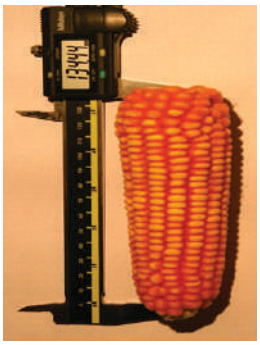
The presence of significant genetic variability, as indicated by the ANOVA, suggests that these morphological traits can serve as reliable selection indices for identifying tolerant genotypes. Similar observations were reported by Zaidi *et al.,* (2003); Kumar *et al.,* (2025c) and Thapa *et al.,* (2025), who highlighted that genotypic variability for morphological and phenological traits under waterlogging conditions can effectively discriminate tolerant and sensitive maize lines.

Visually Assessed DUS Characteristics

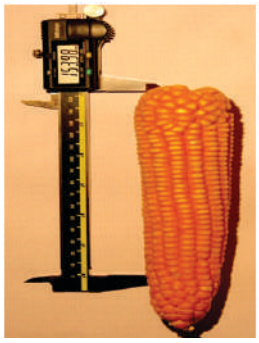
Different visually assessed and measurable traits considered for the experiment were photographed and presented in Fig. 1 and Fig. 2. Data showed significant variation for almost all the traits considered except for the trait, grain type. Since all the maize lines chosen for the experiment were field corn, no entry was recorded for popcorn, waxy corn, opaque corn and sweet corn. The yellow type of colour of grain was recorded for PML 1012, while variegated was recorded for SE 613. I 179



(a) Short



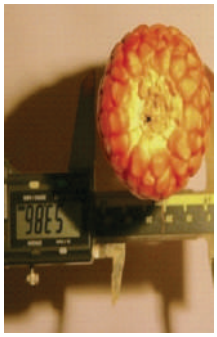
(b) Medium



(c) Long

(d) Small

(e) Medium



(f) Large

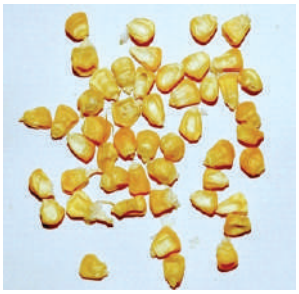
(g) Yellow

(h) White

(i) Red

(j) Orange

(k) Variegated



(l) Shrunken

(m) Round

(n) Indented

(o) Toothed

Fig. 2: Maize inbred lines showing variability for different measurable DUS characters.

Cob length a: short, b: medium, c: long; ear diameter d: small, e: medium f: long; ear shape g: conical, h: cylindrical, i: conical-cylindrical; colour of grain j: yellow, k: white, l: red, m: orange, n: variegated; kernel shape o: shrunken, p: round, q: indented, r: toothed.

and I 202 have red type of grain colour. None of the grain has white or white with a cap state of grain colour. Accordingly, almost every line showed white colouration of glumes of cob except for only two lines, LM 21 and SE 607, which showed light purple colouration. None of the lines showed dark purple colouration. A single line SE 601 showed a shrunken type of kernel shape, while most of the rest were round or toothed, and a few were indented. None of the lines showed a pointed type of kernel shape. These variations are valuable for DUS testing, as visual traits are among the primary descriptors used for establishing varietal distinctiveness (Gethi *et al.,* 2002; PPV&FRA, 2007). Similar patterns of variability for kernel and glume traits have been reported in tropical maize germplasm, emphasizing their importance in genotype differentiation and hybrid parental line identification (Huasheng, 2011; Saha *et al.,* 2024).

Clustering Based on Visually and Measurable Assessed Characters

Twenty different visually assessed and eight different measurable DUS descriptors were employed to generate a Euclidean dissimilarity coefficient matrix, followed by clustering using Ward. D^2 algorithm with the aid of the R Studio software package. The dendrogram thus obtained revealed two major clusters (Fig. 3a). Both Cluster I and Cluster II were further divided into two sub-clusters. Cluster I included the majority of genotypes, consisting of 56 lines, while Cluster II included 24 genotypes. Each sub-cluster of Cluster I was further divided into two major groups, while the first sub-cluster of Cluster II consisted of LM 21 and SE 607, and the second sub-cluster was bifurcated into two major groups. The cluster analysis clustered the

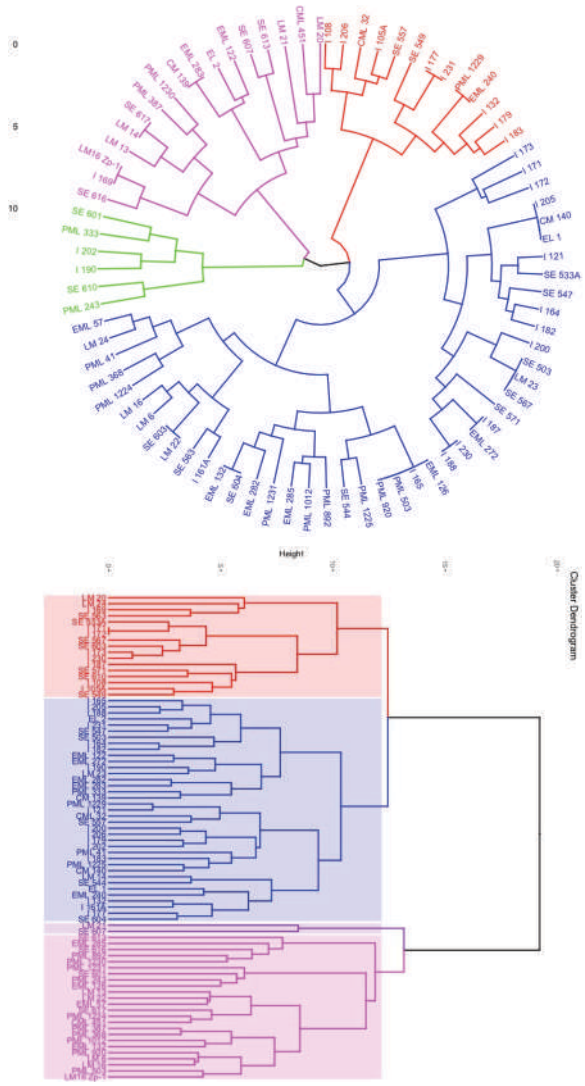


Fig. 3: Clustering of genotypes based on visually assessed and measurable DUS characters.

80 genotypes (Fig. 3b) into two major clusters. Both clusters were further divided into two subclusters. Cluster I consisted of 27 genotypes, while 53 genotypes were included in Cluster II. Each sub-cluster cluster further bifurcated into two major groups. A tanglegram (Fig. 4) was constructed to compare both dendrograms, revealing partial correspondence between visual and measurable trait-based clustering patterns.

Such clustering patterns emphasize that morphological diversity in maize is largely driven by both qualitative and quantitative traits. The clear differentiation of certain lines, such as PML 243, SE 610, SE 601, I 190 and I 202, suggests that they possess unique trait combinations that may serve as reference lines in DUS testing. Similar approaches were employed by Kumari *et al.,* (2025) and Xiao Hong et al. (2006), who used multivariate clustering of maize inbreds based on morphological descriptors to establish distinctness and

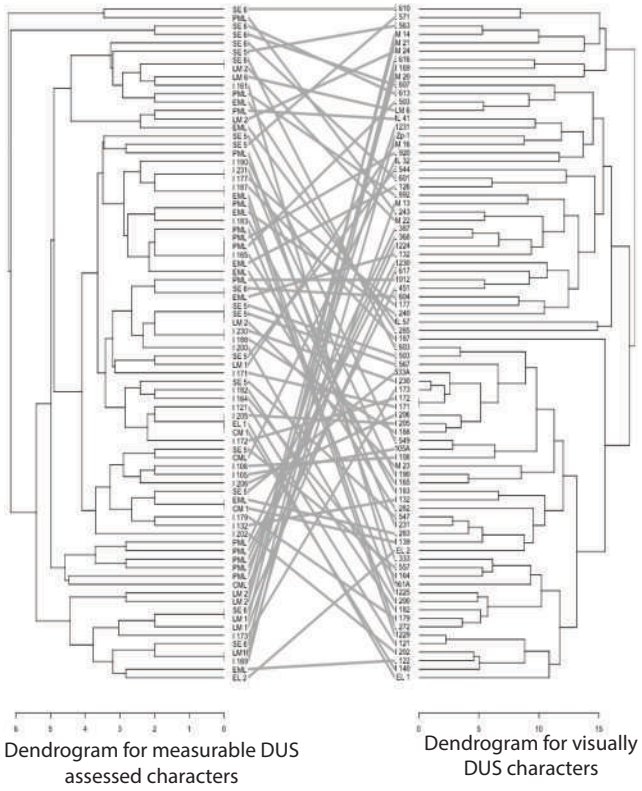


Fig. 4: Tanglegram showing relatedness between different genotypes for measurable and visually assessed DUS characters.

aid in hybrid parent selection.

The observed grouping patterns also align with findings of Tasnim *et al.,* (2025), who reported that measurable and visual DUS traits collectively provide a robust basis for varietal identification and protection under the PPV&FRA framework. The combination of qualitative and quantitative traits thus strengthens the reliability of DUS testing and can contribute to both genetic purity maintenance and stress tolerance breeding, especially under variable environmental conditions such as waterlogging.

Conclusion

The present investigation revealed significant morphological diversity among maize inbred lines evaluated under waterlogging stress using DUS descriptors. Highly significant genotypic differences across measurable and visually assessed traits confirmed the existence of substantial genetic variability. The clustering pattern demonstrated that both qualitative and quantitative traits contribute meaningfully to genotype differentiation. Distinct lines such as SE616, SE610, SE601, I190 and I202 exhibited unique trait combinations, suggesting their potential utility as reference genotypes for DUS testing and as promising parents in waterlogging tolerance breeding.

The integration of DUS characterization with stress-response evaluation thus provides a reliable framework for varietal identification, genetic purity assurance, and sustainable maize improvement under variable environmental conditions.

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RESEARCH ARTICLE

Chimeric Apple: A Study of Morphological and Biochemical Variations

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Abstract

Chimeras are generally derived from spontaneous bud sport mutations in plants whose tissues are made up of two or more types of cells with different genetic makeup. The apple, one of the most well-known fruits, is very prone to these spontaneous mutations or chimeras. In this study, we discovered a half-red and half-green colouring spontaneous apple chimera 'JCS' from the cultivar 'Royal Delicious' in the Kinnaur district of Himachal Pradesh, India. Apple chimera 'JCS' also showed variation in different morphological and biochemical characters as compared to its parent tree. Spontaneous chimeras with different colour combinations may attract consumers and also may be a vital resource for revealing molecular mechanisms, and might be employed in future apple breeding programmes.

Keywords: Apple, Bud sports, Chimera, Conservation, Fruit colour, Genetic variation

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Introduction

The Apple (*Malus* × *domestica* Borkh.) is among the most consumed fruits throughout the world (Kumar *et al.,* 2024a; Kumar *et al.,* 2024b; Sharma *et al.,* 2025). It is a member of the Rosaceae family having chromosome number 2n = 2x = 34, which originated in Central Asia (Kumar *et al.,* 2022a; Kumar *et al.,* 2022b). Apple is known for its various health benefits and helps to reduce the risk of several diseases like diabetes, lung cancer and asthma. It is also rich in flavonols, polyphenols, vitamins and minerals (Preti and Tarola, 2021). Apple breeding for qualitative and quantitative traits is the main concern for fruit breeders due to its nutritional and health benefits (Kumar *et al.,* 2022a). The apple's fruit colour and maturity are the most desirable attributes because they influence the fruit's quality, price, and market acceptance, making the fruit attractive, desirable, and edible for consumers (Goncalves *et al.,* 2017).

Chimeras are generally derived from spontaneous bud sport mutations in plants whose tissues are made up of two or more types of cells with different genetic makeup (Frank and Chitwood, 2016), triggered by genetic variation in somatic cells and such chimeras can give rise to mature tissues of different phenotypes (Chevreau *et al.,* 1989), eventually resulting in numerous phenotypic alterations in qualitative and quantitative traits including variations in fruit colour and

maturity (Kumar *et al.,* 2022a). Trees with chimeras are known for having a mixture of both wild-type and mutant cells (Li, 2024), which produce cultivars like Cytisus Adami and Bizzaria with horticulturally valuable traits (Frank and Chitwood, 2016). Generally, three types of chimeras have been found in Nature (Pratt, 1960) i.e. Periclinal Chimeras (when whole of the L_1, L_2, or L_3 histogenic layer is affected), Mericlinal Chimeras (when onle a part of the meristem layer is affected) and Sectorial Chimeras (when onle a part of the L_1, L_2, or L_3 layer is affected).

Apple, particularly the 'Delicious' group, is prone to these spontaneous mutations or chimeras, for example, the globally known cultivar 'Red Delicious', which is a sport mutant of 'Delicious'. However, old crop improvement methods in apple are laborious and complicated due to the complex nature of the crop. Spontaneous Chimeras are rare, and identification & characterization of such Spontaneous bud sport Chimeras in apple is important as they might be a major source of unique cultivars as well as vital resources for revealing molecular mechanisms (Wu *et al.,* 2021). However, commercial utilization of such spontaneous chimeras requires phenotypic stability still it can be used as a rapid method of breeding in apples (Reichert, 2021, Kumar *et al.,* 2022a) and this can be achieved by either eliminating chimerism by mutagenesis, by selecting a chimeral pattern with an acceptable level of stability and separate chimeras into pure types (Chevreau *et al.,* 1989).

Taking this into account, as well as the increased likelihood of spontaneous apple chimeras at higher elevations, the present investigation was carried out, and a Spontaneous apple chimera 'JCS' was located from Shong village of the district Kinnaur in Himachal Pradesh, India. Fruits of identified chimera and fruits of its parent plants were evaluated for various morphological and biochemical parameters, and bud wood from bud sports was collected, conserved and propagated for further investigations.

Materials and Methods

Experimental locations

To identify apple spontaneous bud sports and chimeras, apple-growing areas were surveyed during this study. An identified chimera in apple was located at Shong village of district Kinnaur in Himachal Pradesh, India (Latitude: 31.44°, Longitude: 78.20°) with an altitude of 2423 metres above mean sea level. However, morpho-biochemical analyses were performed at Dr Yashwant Singh Parmar University of Horticulture & Forestry, Nauni, Solan, Himachal Pradesh, India.

Morpho-metric studies

After the identification of a spontaneous apple chimera, the mother trees as well as the identified chimera were tagged and studied extensively. Standard apple descriptor (IBPGR, 1982) and DUS test guidelines (UPOV, 2005) were followed to record the variations in leaf, flower and fruit characters. However, the bark colour was observed visually and presented as grey and reddish-brown. The LI-COR 3100 leaf area meter was used to measure the leaf area, and the average was displayed in cm^2. A digital pressure tester (FHP-802) was used to determine fruit firmness, i.e. expressed in kg/cm^2.

Fruit colour, overcolour and ground colour were determined using the colour chart of the Royal Horticultural Society. The relative area of fruit overcolour, grooves, lenticels, calyx persistence and. However, the quantum of fruit russet was visually assessed and recorded as 0%, 12%, 25%, 37%, 50%, 62%, 75%, 87% and 100%. Whereas the intensity of russet was visually assessed using standard descriptors and indicated as extremely fine, very fine, intermediate, coarse, scaly and cracked. Fruit volume (cc) and specific gravity were determined through the water-displaced method (Mazumdar *et al.,* 2003). Organoleptic characteristics were assessed by five panellists, who scored the samples using the 'Hedonic Rating Test'.

Biochemical analysis

Biochemical parameters such as total sugars (%), titratable acidity (%), non-reducing sugars (%) and reducing sugars (%) were obtained using the standard method given by Ranganna (1995). While total soluble solids (°Brix) and ascorbic acid content were assessed using the method given by the Association of Official Analytical Chemists. To assess the presence of anthocyanin in apple fruit, the procedure outlined by Herborne (1973) was used. Apple fruits from both the mother plants and the bud sports were investigated for starch iodine stain area, measured using the 'Hedonic Rating Test' scale (Smith *et al.*,1979).

Germplasm conservation

To raise the plants for future studies and conserve the germplasm, bud wood of an identified chimera was collected and propagated on M-9 (T337) rootstock and maintained in the fruit nursery of the Dr YS Parmar University of Horticulture & Forestry, Nauni, Solan, Himachal Pradesh, India.

Statistical analysis

The experimental data for each parameter under study were statistically analysed. For morphometric and biochemical analysis, three replications were used. Results from spontaneous chimera and mother plant were compared, and data were collected in triplicate and presented as the mean ± standard error of the mean.

Results and Discussion

Exploration of existing apple orchards led to the identification of a half-red and half-green colouring apple chimera 'JCS' originating from 'Royal Delicious,' which can be used to exploit variability and new genotypes with one or more commercially useful traits. Extensive phenological, floral, and fruit evaluations were conducted on the spontaneous apple chimera 'JCS', and its parent is detailed in Table 1. Here, we reveal and discuss the results of our study.

Fig. 1: Identified half red and half green colouring apple chimera 'JCS' and their parent cultivar 'Royal Delicious'.

Spontaneous apple chimera 'JCS' was primarily identified and isolated for a distinct half red and half green colouring pattern with respect to its mother cv. Royal Delicious is located at Shong, district Kinnaur (Latitude: 31.44°, Longitude: 78.20°). Apart from its unique colouring pattern, spotted and varied in fruit shape, which was cylindrical compared to conic fruits of the mother tree (Fig. 1).

Half red and half green colouring apple chimera 'JCS' also showed variation in different characters and revealed higher fruit weight (138.00 g), fruit breadth (6.37 cm), fruit base cavity (eye) length (1.22 cm), fruit base (eye) cavity depth (1.25 cm), juice content (56.00%), titratable acidity (0.21%), TSS (12.10 ° Brix), total sugars (5.56%), non-reducing sugars (2.76%), ascorbic acid/ vitamin – C (11.20 mg/100 g), anthocyanin content (0.39 OD), fruit firmness (12.00 kg/cm^2), fruit peel lenticels (6 per cm^2), lesser stained area (70%). Less fruit length (6.79 cm) and pedicel length (1.67 cm), leaf area (26.00 cm^2), reducing sugars (2.66 %), fruit cavity depth (1.32 cm), number of seeds (5), and relative area of overcolour (50.00 %) were observed. Other characters examined were more or less similar in parent tree and spontaneous apple chimera 'JCS' (Table 1).

Table 1: Phenological, flower, fruit, morpho-physical and biochemical characters of half red and half green colouring apple chimera 'JCS' and their parent cultivar 'Royal Delicious'

Location: Shong, Kinnaur (Latitude: 31.44°, Longitude: 78.20°) (Altitude: 2423 m)		
Characters	**Parent Cultivar: Royal Delicious**	**Bud Sport: JCS**
Plant morphological characters		
Bark colour	Grey	Grey
Internodal length (cm)	2.50	Yet to be ascertained
Phenological characters		
Time of leaf bud burst	1st week of March	1st week of March
Time of floral bud burst	3rd week of March	3rd week of March
Time of peel colour development	4th week of July	4th week of July
Time of leaf fall	3rd week of December	3rd week of December
Foliage characters		
Colour of young leaf	Light Green	Light Green
Leaf pubescence	Medium	Medium
Leaf margin	Serrate type 1	Serrate type 1
Leaf apex shape	Acuminate	Acuminate
Leaf base shape	Acute	Acute
Leaf area (cm^2)	33.00	26.00

Inflorescence and flowering characters		
Bearing habit	On spurs and long shoots	
Yet to be ascertained Flower colour	Pinkish white	Pinkish white
Season of flowering	Early	Early
Duration of flowering	12-15 days	Yet to be ascertained
Morpho-physical fruit characters		
Fruit weight (g)	121.50	138.00
Fruit length (cm)	6.82	6.79
Fruit breadth (cm)	6.19	6.37
Fruit volume (cc)	141.50	180.00
Specific gravity	0.86	0.77
Fruit firmness (kg/cm^2)	10.50	12.00
Fruit shape	Conic	Cylindrical
Fruit shape index	1.10	1.07
Fruit texture	Intermediate	Intermediate
Fruit ribbing	Weak	Weak
Number of grooves	Moderate and 5 in number	Moderate and 5 in number
Surface of fruit	Smooth	Smooth
Fruit colour	Red group 45-A	Red group 45-A
Fruit overcolour	Red group 45-A	Red group 45-A
Relative area of fruit overcolour (%)	80.00	50.00
Type of overcolour	Striped	Striped
Intensity of fruit overcolour	Medium	Medium
Fruit ground colour	Yellow green group 149 B	Yellow green group 149 B
Stripes (Present/Absent)	Present	Absent
Width of stripes	Medium	Medium
Lenticels	Few	Medium
Fruit peel lenticels (per cm^2)	3	6
Number of locules	5	5
Fruit: aperture of locules	Moderately open	Moderately open
Persistency of calyx	+	+
Pedicel length (cm)	1.75	1.67
Pedicel breadth (cm)	0.43	0.30
Fruit cavity depth (cm)	1.53	1.32
Fruit base cavity (eye) length (cm)	1.04	1.22
Fruit base cavity (eye) depth (cm)	1.07	1.25
Flesh colour	Greenish	Greenish
Pulp texture	5	5
Number of seeds/fruit	6	5
Seed colour	Light brown	Light brown
Average seed weight (g)	0.048	0.052
Harvest time	1st week of September	1st week of September
Organoleptic analysis		
Pulp texture	5	5
Pulp taste	5	5
Pulp juiciness	5	5
Fruit attractiveness	Intermediate	Intermediate
Eating quality (dessert)	Intermediate	Intermediate
Visual observation		
Russeting (%)	Nil	Nil
Russet type	Absent	Absent

Biochemical parameters		
Titratable acidity (%)	0.18	0.21
Total soluble solids (°Brix)	11.80	12.10
Total sugars (%)	4.96	5.56
Reducing sugars (%)	3.21	2.66
Non-reducing sugars (%)	1.66	2.76
Juice content (%)	55.00	56.00
Anthocyanin content (A_{530})	0.37	0.39
Ascorbic acid / Vitamin C (mg/ 100 g)	10.55	11.20
Starch content (%)	75.00	70.00

Now days farmers are adapting to new cultivars with earlier and more intense colour, to increase market price. The fruit colour and maturity in apples are the foremost desirable attributes because they influence the fruit's price and market acceptance. However, spontaneous bud sports and chimeras with different colours or colour combinations may attract consumers, and also, such spontaneous bud sports or chimeras might be a major source of unique cultivars as well as vital resources for revealing molecular mechanisms and might be employed in future apple breeding programmes.

A huge number of spontaneous chimeras are already found in different flower and fruit crops like pear (Chevreau *et al.,* 1989), and some chimeras of apple were reopted from the diploid apple cultivars 'Ontario', 'Wealthy', 'Jona-than' and 'Rome' (Blaser and Einset, 1948). Whereas, the structure of chimeras in the carnation and apple cultivar 'Giant Spy' has been modified through X-rays (Pratt, 1960). Initial chimerism in perennial trees can be efficiently reduced through regeneration (Malabarba *et al.,* 2020). Cytological study of diploid-tetraploid apple cytochimeras revealed that cytochimeral variation was caused by layer replacement, and apple cytochimeras were shown to be more stable under normal conditions (Pratt *et al.,* 1967). Periclinal chimera can be propagated through cuttings as they most stable due to completely colonising a cell layer (Sichel *et al.,* 2023). However, Graft chimeras may have the potential to improve the growth of plants, quality of fruits, and disease resistance in plants (Augstein and Melnyk, 2025).

The findings of the present study revealed variation in morphological and biochemical compositions of spontaneous chimera, and such type variations in chimera and their parents are quite common. The maturation of apples is observed by texture, firmness, starch index, soluble solids and titratable acidity (Goncalves *et al.,* 2017). Fruit ripening and maturity influence fruit colour, texture, and aroma (Su *et al.,* 2025). This may be due to genetic changes or might be due to epigenetic modifications, which impact fruit ripening in apple (Kumar *et al.,* 2024c). Variations in the shape of chimera correspond to the results of Michelangelo *et al.,* (2013) and Kumar *et al.,* (2022a), who previously documented changes in the shape of the fruits in bud sports. However, Liu *et al.,* (2003) discovered a bud sport called 'Hongjiangjun' with a soluble solids content of 13.9-15.8%, a pleasant acid-sweet flavour, and excellent eating quality. The spur bud sport mutant of 'Nagano Fuji 2' resulted in the improvement of a new apple known as 'Tianhong 2' with a 14.5-16.8% of soluble solid content, a smooth finish, and a strong fragrance (Shao *et al.,* 2008). Similarly, Wang *et al.,* (2010) discovered 'Pinkish Qinguan' as a sport (mutant) of the Qinguan apple cultivar, with large fruits with a pretty pinkish colour on the surface, yellow-white flesh, fine, tender, crisp and juicy texture, having soluble solids 16%, and a very decent eating quality.

Germplasm collection and conservation

To raise the plants for future studies and conserve the germplasm, bud wood of the identified apple chimera was collected and propagated on M-9 (T_{337}) rootstock, and the germplasm was conserved to check the stability of the changed traits.

Conclusion

Genetic improvement in apple through traditional methods is complicated and time-consuming. The fruit colour and maturity are the foremost desirable attributes in apples, as they influence the price and market acceptance. Nowadays, farmers are adapting to new cultivars with earlier and more intense colour, to increase market price. Spontaneous bud sports and chimeras with different colours or colour combinations may attract consumers, and also, such spontaneous bud sports or chimeras might be a major source of

unique cultivars as well as vital resources for revealing molecular mechanisms and might be employed in future apple breeding programmes. However, commercial utilization of such spontaneous chimeras requires phenotypic stability, which can be achieved by either eliminating chimerism by mutagenesis, separating chimeras into pure types or selecting a chimeral pattern with an acceptable level of stability.

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RESEARCH ARTICLE

Economic, Social, and Cultural Factors Influencing On-farm Mango (*Mangifera indica*) Diversity Conservation in Uttar Pradesh

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Abstract

This study examines the conservation practices employed by mango custodian farmers in Uttar Pradesh, specifically targeting those who cultivate a minimum of ten mango varieties in their orchards. Data were collected on various motivational factors (personal, social, economic, cultural, environmental and policy) influencing on-farm mango diversity conservation from 101 mango farmers in 23 districts of India. Results indicated that economic and personal incentives were the most driving factors for the farmers to conserve and maintain mango diversity. This research underscores the essential contribution of custodian farmers to the conservation of mango diversity and the necessity for supportive policies that acknowledge farmers' roles in these conservation initiatives. Comprehending these motivational factors enables stakeholders to formulate effective strategies for improving on-farm biodiversity conservation in mango cultivation regions of Uttar Pradesh.

Keywords: Custodian farmer, Diversity conservation, Mango, Motivational factor

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Introduction

Mango (*Mangifera indica* L.) is a paramount fruit crop in India, holding substantial cultural and economic importance. Uttar Pradesh, especially areas such as Malihabad, is distinguished for its vast mango orchards, which significantly bolster the state's agricultural economy (Yadav and Rajan, 1993; Rajan *et al.*, 2011; 2024). The preservation of traditional mango varieties encounters escalating challenges due to urbanization, alterations in cropping patterns, and economic factors. The motivation of farmers to preserve these varieties is shaped by a complex interaction of economic, social, cultural, environmental, and policy-related factors (Mal *et al.*, 2010; Gajanana *et al.*, 2015). Custodian farmers are essential for the conservation and propagation of traditional agricultural varieties on farms, greatly influencing biodiversity and sustainable agriculture. In Uttar Pradesh, especially in its varied mango cultivation areas, these farmers act as custodians of the state's abundant mango heritage, diligently preserving multiple indigenous mango varieties (Rajan *et al.*, 2013; 2015). Custodian farmers engage in more than simple cultivation; they employ Indigenous Technical Knowledge (ITK) to preserve and augment the genetic diversity of mango species, demonstrating a profound connection to their agricultural

practices and local ecosystems (Sthapit *et al.*, 2013; Rajan *et al.*, 2024).

A thorough survey conducted in 23 districts rich in mango diversity in Uttar Pradesh highlighted the significant contributions of 101 custodian farmers who collectively preserve various mango types, illustrating the district-specific differences in cultivation practices and fruit attributes (Rajan *et al.*, 2024). The motivations of these farmers include self-interest, social prestige, and the desire to conserve unique varieties, as emphasized by Dinesh *et al.,* (2015), which highlights the influence of personal and cultural ties on agricultural practices.

Malihabad region of UP upholds a strong tradition of mango conservation, owing to traditional agricultural practices, the proficiency of nursery experts, community engagement, and economic viability. Heritage orchards have been maintained, nursery specialists have been involved to conserve genetic diversity, and participatory rural appraisal tools have also been employed to enhance mango diversity in the region. In addition, the cooperation of the community with agri-experts leads to new conservation plans that combine scientific technology and traditional wisdom (Ram and Rajan, 2003; Rajan *et al.*, 2015).

Materials and Methods

Study Area

This study was conducted across major mango-growing regions in 23 districts of Uttar Pradesh, India (Table 1). These districts were chosen for their significance in mango diversity and production, which represent a variety of agro-climatic conditions conducive to mango cultivation. Surveys were executed in principal mango-producing regions within these districts to evaluate the conservation of on-farm mango diversity and to comprehend the factors incentivizing farmers to serve as custodians of traditional mango varieties

Data Collection Framework

The data collection process aimed to identify the motivations influencing farmers' decisions to maintain mango diversity, employing six key dimensions based on the framework developed by Padulosi (2011). The examined drivers are listed in Table 1. The dimensions included personal drivers, encompassing intrinsic motivations such as a passion for mango cultivation, hobby farming, familial preferences, and an awareness of the significance of conserving diversity for future generations. Social drivers encompassed community-oriented motivations, such as the aspiration to safeguard local agricultural heritage, disseminate planting materials among friends and relatives, and sustain a diverse mango collection. The economic drivers emphasized the significance of diversity in enhancing economic resilience, offering a variety of marketable alternatives, and alleviating risks associated with fluctuating yields or prices. Cultural factors demonstrated the acknowledgement of the relationship between agricultural biodiversity and cultural identity, traditions, and customs. Environmental factors highlighted the significance of biodiversity in enhancing ecosystem services, advancing soil and plant health, and fostering sustainable agricultural practices. Finally, policy-related factors examined the impact of governmental policies, programs, or institutional support systems that promote *in-situ* conservation of agrobiodiversity. The data collection process sought to ascertain the motivations affecting farmers' choices to preserve mango diversity, utilizing six principal dimensions derived from the framework established by Padulosi (2011) and adapted from Rajan *et al.,* (2024). The drivers studied are mentioned in Table 1. These dimensions included personal drivers, which encompassed intrinsic motivations such as a passion for mango cultivation, hobby farming, family preferences, and an awareness of the importance of preserving diversity for future generations. Social drivers included community-

Table 1: Selected districts, mango-growing areas, and custodian farmers involved in mango diversity conservation

S.N.	District	Blocks/areas	Custodian farmer
1.	Aligarh	Billona (Billona Chitrasi)	Asif Khan, Mehandi Hasan
2.	Amethi	Thauri	Anant Bahadur Singh
3.	Amroha	Sihali Jagir (Block -Gajraula), Salempur (Hemnagar), Keshavpur, Sekhupura Jhakari	Sabahat Ali Khan, Wazahat Ali Khan, Mohammad Suleman, Bijay Kumar Yadav, Santosh Kumar
4.	Bagpat	Rataul	Junaid Faridi, Sahabuddin Ahamad Siddiqui, Abdul Kalam

5.	Balrampur	Chirraiya, Phulveria Birahimpur	Sadhu Ram Maurya, Rajeshwar Pratap Singh
6.	Bulandsahr	Alipur (Pahanshu), Bharkaow (Block- Unchagaon, Tehsil- Siyana), Bhoranshi (Nawab Chhatari, Block- Pahanshu), Patti Hazari (Siyana), Siyana	Firoz Mohammad Khan, Hazi Jameel, Narendra Kumar Tyagi, Anil Tyagi, Abhay Narayan Singh
7.	Faizabad	Sariyawa, Masodha	Tej Bahadur Singh
8.	Gonda	Pure Chain Kunwar, Sahjanwa-Rupaideeh	Anil Chandra Pandey
9.	Jaunpur	Mai (Buxa), Barauli (Badlapur), Kuddupur, Purew	Shashikant Yadav, Triveni Singh, Dinesh Singh
10.	Kannauj	Gadanpur Baddu	Ashwini Kumar Pathak, Om Prakash Tiwari
11.	Kasganj	Mahawar, Dehari, Nagla Amber (Inayati), Lodhipur	Brajendra Singh, Ganga Singh, Ram Naresh Yadav, Hem Singh
12.	Lakhimpur Khiri	Hindustan Farm	Nirmal Singh
13.	Lucknow	Gopramau, Sarsanda, Kasmandi Kalan, Mohammed Nagar Talukedari, Nabipanah, Nadan Mahal Road, Malihabad, Narauna, Tiwari Kheda, Naubasta, Mall, Gosaiganj, Godwa, Para Bandrahi	Kamla Devi, Mahadin, Moin Ashraf, Monish Ahemad, Muneshwar, Narendra Shukla, Ram Asre Sharma, Sunil Kumar, Parmeshar Sharma, Ram Swaroop, Vidyawati, Ram Naresh, Ajajul Hasan, Mahesh Kumar, Ram Kishor, Chhote Lal Kashyap, Maiku Lal, Nawab Hasan, Upendra Kumar Singh, Abdul Samad, Sadab Ali, S. C. Shukla, Haji Kalimullah Khan, Durga Prashad, Iqbal Ahmad, Kuldeep Kumar, Mandhvendra Deo Singh, Md Iliyas, Ram Pal Maurya, Sanjay Singh, Shiv Kumar Dwivedi, Utkarsh Singh Chauhan, Ajeet Shukla
14.	Mau	Galib Pur, Muhammadabad Gohna	Abdul Salam Khan
15.	Meerut	Kithore	Mustafa, Ragib Ali, Mumtaz Ali, Sarafat Ali
16.	Muzaffarnagar	Pinna, Purkazi	Shiv Narayan, tariq Mushtafa
17.	Saharanpur	Behat, Kheda Afgan, Nandi Firojpur,Toli, Kashipur	Ajai Jain, Amir Khan, Azam Khan, Khairati Lal, Seth Pal Singh, Satya Kumar, Ramvir Singh Chauhan, Tafzeel Ahmad
18.	Sant Kabir Nagar	Harraiya	Akhand Pratap
19.	Shamli	Babri, Gazipur	Shri Somansh Prakash, Raghav Swaroop, Ali Mia
20.	Shravasti	Labedpur	Pankaj Deo Singh
21.	Siddharthanagar	Dhanghata	Ram Swarth Mishra
22.	Sitapur	Achra Mau, Barsandhiya, Nighuwa Mau, Rehamabad, Dharwa Garhi, Revankala Garhi, Kala Bagahiya, Kesharipur Kothar, Sita Rasoi- Sidhauli, Indrauli Maholi Block, Ambarpur Biswan, Biswa	Mohd. Ayaz, Sankar Bakhs Singh, Awadhesh Birbal, Mohd. Farid Khan, Mohd. Rafik, Vishal Singh, Dr Yash Pal Singh, Deepak Yadav, Indu Shukla, Bhanu Pratap Singh, Naresh Pal Singh, Md. Haneef, Md. Tafzeel
23.	Varanasi	Chiraigaon, Barbaspur, Barai-Umaraha, Sultanpur, Pilkhini, Kibli Bhtsar	Aditya Narayan Maurya, Kaushal Kumar Maurya, Subhash Chandra Pandey, Anil Singh, Akhilesh Narayan Singh

Table 2: Motivational factors influencing custodian farmers in the maintenance of diverse mango varieties

S.N.	Custodian farmers	Varieties maintain	Orchard size (ha)	Average orchard age (years)	Motivation	Source of motivation
1.	Asif Khan	13	2.4	22	SELF, SOIN	PSNL, ECON
2.	Mehandi Hasan	39	4.4	30	NEXT, SELF, SOIN	SOCL, PSNL, ECON
3.	Anant Bahadur Singh	11	1	30	SELF, IDMO, ATMA	PSNL
4.	Sabahat Ali Khan	21	15	60	IDMO, ATMA, SELF, SOIN	PSNL, ECON
5.	Wazahat Ali Khan	13	15	30	SELF, SOIN	PSNL, ECON
6.	Mohammad Suleman	22	13.5	40	SELF, ATMA, ANCP	PSNL, ECON, SOCL
7.	Bijay Kumar Yadav	11	0.8	30	SELF, SOIN, ATMA, SOPR	PSNL, ECON, SOCL

8.	Santosh Kumar	12	5	20	SOIN, SELF, ENVN	ECON, PSNL, ENVM
9.	Junaid Faridi	27	12	80	SELF, SOIN	PSNL, ECON
10.	Sahabuddin Ahamad Siddiqui	25	12	100	ANCP, SOPR, SOIN	SOCL, ECON
11.	Abdul Kalam	105	5	60	SELF, ATMA, SOIN	PSNL, ECON
12.	Sadhu Ram Maurya	12	4	50	SOIN, SELF	ECON, PSNL
13.	Rajeshwar Pratap Singh	31	2.5	10	SELF, COLM, SOIN	PSNL, ECON
14.	Firoz Mohammad Khan	17	25	50	ANCP, SELF, SOIN	SOCL, PSNL, ECON
15.	Hazi Jameel	20	22	250	ANCP, FPRO, SOIN	SOCL, PSNL, ECON
16.	Narendra Kumar Tyagi	12	2.8	18	SELF, SOIN	PSNL, ECON
17.	Anil Tyagi	12	38	50	SOIN	ECON
18.	Abhay Narayan Singh	17	103	130	ANCP, SOIN, SOPR	SOCL, ECON
19.	Tej Bahadur Singh	11	12	50	SOIN	ECON
20.	Anil Chandra Pandey	10	0.4	20	SELF, SOIN	PSNL, ECON
21.	Shashikant Yadav	10	3.75	200	ANCP	SOCL
22.	Triveni Singh	11	22.6	35	SOIN, SUSE, DAFR	ECON, PSNL, SOCL
23.	Dinesh Singh	10	1	60	SOIN, SUSE	ECON, PSNL
24.	Ashwini Kumar Pathak	55	23	50	ANCP, SELF, SOIN	SOCL, PSNL, ECON
25.	Om Prakash Tiwari	12	10.5	75	SELF, SOIN	PSNL, ECON
26.	Brajendra Singh	11	2.4	47	SOIN, SELF, SOPR	ECON, PSNL, SOCL
27.	Ganga Singh	13	9	35	SOIN, SELF, SOPR	ECON, PSNL, SOCL
28.	Ram Naresh Yadav	20	2	40	SOIN, SELF, SOPR	ECON, PSNL, SOCL
29.	Hem Singh	13	2.4	50	SOIN, SELF	ECON, PSNL
30.	Nirmal Singh	10	2	16	SELF, SOIN, ATMA, SOPR	PSNL, ECON, SOCL
31.	Kamla Devi	25	2.25	25	SOIN, SUSE, DIVF	ECON, PSNL
32.	Mahadin	38	0.38	30	SOIN, SUSE	ECON, PSNL
33.	Moin Ashraf	25	1.25	35	SOIN	ECON
34.	Monish Ahmad	28	1.5	30	SOIN	ECON
35.	Muneshwar	25	0.75	35	SOIN	ECON
36.	Narendra Shukla	40	2.25	40	SOIN, SUSE, DAFR, SOPR	ECON, PSNL, SOCL
37.	Ram Asre Sharma	25	1	41	SOIN, SUSE	ECON, PSNL
38.	Sunil Kumar	21	0.75	35	SOIN, SUSE, DAFR	PSNL, ECON, SOCL
39.	Parmeshar Sharma	44	1	30	SOIN, AREA, SUSE	ECON, ENVM, PSNL
40.	Ram Swaroop	18	1.5	31	SOIN, SUSE, AREA, ATMA, DAFR	ECON, PSNL, ENVM, SOCL
41.	Vidyawati	15	1.25	25	SOIN	ECON
42.	Ajajul Hasan	12	1.25	65	SOIN, SUSE	ECON, PSNL
43.	Mahesh Kumar	12	0.5	30	SOIN, SUSE	ECON, PSNL
44.	Ram Kishor	10	0.63	42	SOIN, SUSE	ECON, PSNL
45.	Chhote Lal Kashyap	138	2	40	IDMO, SOIN, COLM	PSNL, ECON, SOCL
46.	Maiku Lal	42	3	35	SELF, IDMO, COUV, COLM	PSNL, ECON
47.	Nawab Hasan	51	1.5	75	SOIN, ATMA, ANCP, COUV	SOCL, PSNL, ECON
48.	Upendra Kumar Singh	19	5.5	35	ATMA, COLM, DIVF	PSNL, SOCL
49.	Abdul Samad	18	3.5	60	COLM, ANCP, ATMA, SELF, COLM	PSNL, SOCL
50.	Sadab Ali	50	7.2	25	SELF, SOIN, ATMA, SOPR	PSNL, ECON, SOCL
51.	S. C. Shukla	272	25	30	MGOV, SELF, DIVF	SOCL, PSNL
52.	Haji Kalimullah Khan	53	5.5	100	SELF, ATMA	PSNL
53.	Durga Prashad	11	1.5	50	SELF, SOIN	PSNL, ECON
54.	Iqbal Ahmad	13	2.5	55	SOIN, ANCP, AREA, SELF	ECON, ENVM, PSNL
55.	Kuldeep Kumar	11	1	30	ANCP, SOIN	SOCL, ECON
56.	Mandhvendra Deo Singh	80	20	100	ANCP, AREA, ATMA, SELF, COLM, COUV	SOCL, ENVM, PSNL

57.	Md Iliyas	10	0.25	100	SOIN, SELF	ECON, PSNL
58.	Ram Pal Maurya	11	0.25	30	SELF, SOIN	PSNL, ECON
59.	Sanjay Singh	15	8	40	SELF, SOIN	PSNL, ECON
60.	Shiv Kumar Dwivedi	17	12	30	ANCP, COLM, SOIN	SOCL, PSNL, ECON
61.	Utkarsh Singh Chauhan	14	4	35	SOIN, AREA, SELF	ECON, ENVM, PSNL
62.	Ajeet Shukla	17	12.5	40	SELF, DIVF, COUV, NEXT	PSNL
63.	Abdul Salam Khan	24	1	30	SELF, SOIN, ATMA	PSNL, ECON
64.	Mustafa	19	25	50	ANCP, SOIN	SOCL, ECON
65.	Ragib Ali	11	2	40	SOIN	ECON
66.	Babu Mumtaz Ali	20	42	60	ATMA, ANCP, SOPR	PSNL, SOCL
67.	Sarafat Ali	24	51	45	ANCP, SELF	SOCL, PSNL
68.	Shiv Narayan	18	25	75	SELF, SOIN	PSNL, ECON
69.	Tariq Mustafa	104	29	75	ANCP, SELF, SOIN	SOCL, PSNL, ECON
70.	Ajai Jain	40	154	60	SELF, SOIN	PSNL, ECON
71.	Amir Khan	57	65	80	SELF, SOIN, SUSE, DAFR	PSNL, ECON, SOCL
72.	Azam Khan	36	51	55	SOIN, SELF, SOPR, EXOM	ECON, PSNL, SOCL
73.	Khairati Lal	24	11	60	SELF, SOIN, SOPR	PSNL, ECON, SOCL
74.	Seth Pal Singh	14	2.5	25	SOIN, SELF, DIVF, MRKT	ECON, PSNL
75.	Satya Kumar	16	28	60	SELF, DIVF, COLM, SOIN	PSNL, ECON
76.	Ramvir Singh Chauhan	77	102	40	PLFS	RULE
77.	Tafzeel Ahmad	12	7.5	40	SOIN, AREA, SELF	ECON, ENVM, PSNL
78.	Akhand Pratap	12	16	20	SELF, MGOV	PSNL, SOCL
79.	Somansh Prakash	16	128	60	SELF, AREA	PSNL, ENVM
80.	Raghav Swaroop	16	16	60	SELF, SOIN	PSNL, ECON
81.	Ali Mia	19	64	50	ANCP, SELF, ATMA, SOIN	SOCL, PSNL, ECON
82.	Pankaj Deo Singh	35	14	5	SOIN, SELF, IDMO, COLM, COUV, DIVF	ECON, PSNL
83.	Ram Swarth Mishra	10	0.4	20	SELF, ATMA, SOIN	PSNL, ECON
84.	Mohd. Ayaz	13	0.8	40	SOIN, SUSE	ECON, PSNL
85.	Sankar Bakhs Singh	13	0.6	20	SOIN, SUSE	ECON, PSNL
86.	Awadhesh Birbal	14	3.2	60	SOIN, SUSE, SOPR	ECON, PSNL, SOCL
87.	Mohd. Farid Khan	11	1.21	40	SOIN, SUSE	ECON, PSNL
88.	Mohd. Rafik	12	0.41	18	SOIN, SUSE	ECON, PSNL
89.	Vishal Singh	10	10	40	IDMO, SOIN, SUSE	PSNL, ECON
90.	Dr Yash Pal Singh	15	6	22	SOIN, SUSE, SOPR	ECON, PSNL, SOCL
91.	Deepak Yadav	12	0.8	50	SOIN, SUSE	ECON, PSNL
92.	Indu Shukla	12	1.2	50	SOIN, SUSE	ECON, PSNL
93.	Bhanu Pratap Singh	21	3.2	150	SOIN, SUSE	ECON, PSNL
94.	Naresh Pal Singh	17	4.5	60	SELF, ANCP, COLM	SOCL, PSNL
95.	Md. Haneef	12	4	40	SELF, SOIN	PSNL, ECON
96.	Md. Tafzeel	57	30	50	ANCP, SOIN	SOCL, ECON

97.	Aditya Narayan Maurya	11	1.1	50	SOIN, SUSE, FFTM	ECON, PSNL
98.	Kaushal Kumar Maurya	10	1	60	SOIN, SELF	ECON, PSNL
99.	Subhash Chandra Pandey	10	0.5	100	SOIN, SUSE	ECON, PSNL
100.	Anil Singh	11	4	60	SOIN, SUSE, DAFR	ECON, PSNL, SOCL
101.	Akhilesh Narayan Singh	10	1.2	70	SOIN, SUSE	ECON, PSNL

Motivation = ANCP (as a heritage, ancestral property, conserving as a parental property, old family orchard), AREA (mango growing area), ATMA (attachment and love for mango), COLM (collection of mango varieties, genetically divers varietals collection of trees, interest to develop orchard based on seedlings or some lesser-known varieties, to increase the number of mango varieties in orchard), COUV (conservation for the unique varieties, maintenance of available varieties in the orchard, conserve mango varieties for next generation), DAFR (distribution among friends and relatives), DIVF (motivated by mango diversity fair), ENVN (environmental friendly), EXOM (export), FFTM (wood for cooking, furniture etc.), FPRO (family profession), IDMO (interested to develop mango orchard), MGOV (motivated by government institutions i.e. ICAR-CISH, Lucknow and State Horticulture Department), MRKT (good market of mango), NEXT (established orchard for next generation), PLFS (prevent land from sealing), SELF (self interest), SOIN (source of income), SOPR (social prestige), SUSE (self use). Source of Motivation = ECON (economic), ENVM (environment), PSNL (personal), RULE (policy/rule), SOCL (social)

oriented motivations, such as the aspiration to safeguard local agricultural heritage, disseminate planting materials among friends and relatives, and sustain a diverse mango collection. Economic factors underscored the importance of diversity in bolstering economic resilience, offering a variety of marketable alternatives, and alleviating risks associated with fluctuating yields or prices. Cultural factors demonstrated the acknowledgement of the relationship between agricultural biodiversity and cultural identity, traditions, and customs. Environmental factors highlighted the significance of biodiversity in enhancing ecosystem services, augmenting soil and plant health, and fostering sustainable agricultural practices. Finally, policy-related factors examined the impact of governmental policies, programs, or institutional support systems that promote *in-situ* conservation of agrobiodiversity.

Survey Methodology

We conducted a structured questionnaire at each district to identify qualitative and quantitative data about motivational factors in key mango-producing regions. This study explores the motivations of traditional mango variety maintainers and their perceived importance of biodiversity through farmer interviews.

Sampling Framework

To ensure district representation, a multistage sampling method was employed. Experienced mango experts had taken special care in the selection of mango villages in each district. Random samples of farmers were then drawn from within each village to gather detailed data on conservation techniques and motivation drivers.

Data Analysis

In addition to motivational factors, information was gathered on unique mango varieties maintained by farmers based on farmer descriptions. Data included traits such as fruit maturity period, fruit quality (e.g., table fruit or pickling), peel colour, pulp colour, fibre content, and fruit size. We analyzed the collected data using descriptive statistics to assess trends in motivational factors across districts. Motivations were framed as personal (SELF), social (SOIN), economic (SUSE), cultural (ANCP), environmental (ENVN), and policy-related (MGOV), and each classification was plotted in a frequency distribution to assess their relative importance as drivers of conservation.

Results and Discussion

Summary of the motivational factors for custodian farmers in conserving mango varieties presented in Table 2 revealed multiple drivers for custodian farmers ranging from economic, social, religious, ethnic and biodiversity interests. Self-interest (SELF, 55%) and a stable source of income (SOIN, 85%) were found to be the most important and frequent motivation factors among the farmers (Fig.1). The farmers were likely to conserve mango varieties if they had private benefits

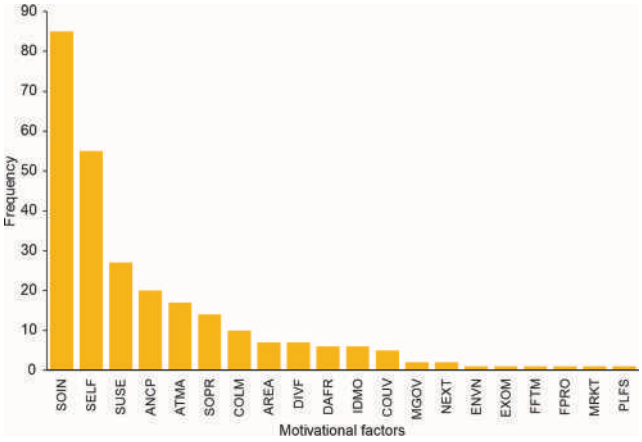

Fig. 1: Frequency of responses for various motivational factors driving on-farm mango conservation.

and economic sustainability. Despite the lesser mention, sustainable use (SUSE, 27%) is also highly relevant, which indicates farmers' priority on resource sustainability through time. Social prestige (SOPR, 14%), which indicates farmers' tendency to conserve partly because they want to be appreciated by the community, and attachment and love for mango (ATAM, 17%), which indicates that farmers have cultural and emotional attachment to the mango cultivation. These were the most notable motivational factors.

External factor-related motivations, including government support (MGOV), export potential (EXOM), market opportunities (MRKT), and environmental concerns (ENVN), were negligible, with frequencies of 2, 1, 1, and 1, respectively. This indicates that these factors are not significant determinants for the majority of farmers. Likewise, motivations such as the establishment of orchards for future generations (NEXT) and the prevention of land sealing (PLFS) were mentioned by only a limited number of farmers. Practical applications such as wood for cooking or furniture (FFTM) were infrequently referenced.

The examination of the motivational factors influencing custodian farmers who cultivate various mango varieties uncovers several significant insights. Farmers maintain between 10 and 272 mango varieties, with notable instances such as S.C. Shukla, who preserves 272 varieties, and Ramvir Singh Chauhan, who has 77 varieties. This indicates a robust dedication to conservation among these farmers. Orchard sizes exhibit significant variation, ranging from a mere 0.25 hectares (Md Iliyas) to an expansive 154 hectares (Ajai Jain), suggesting a relationship between land availability and the diversity of mango varieties cultivated. Orchard ages vary from 5 years (Pankaj Deo Singh) to an impressive 250 years (Hazi Jameel), representing both newly established and heritage orchards of considerable cultural and historical significance.

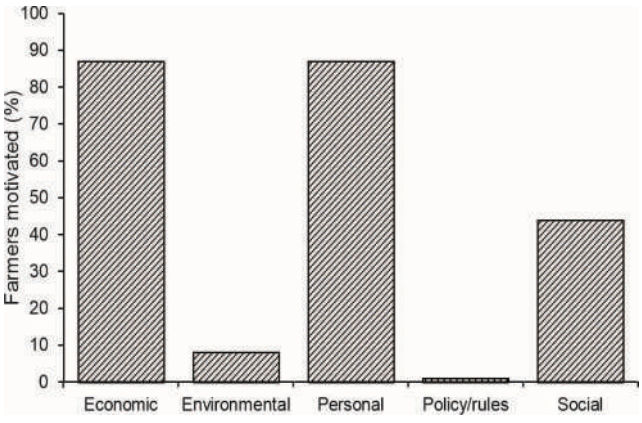

Fig. 2: Relative importance of motivational factors to the custodian farmers for the conservation of mango varieties.

A trend has emerged indicating that economic incentives are associated with larger orchard sizes and an increased number of cultivated varieties. Farmers motivated by ancestral land frequently oversee mature orchards, highlighting the cultural importance of mango cultivation in heritage preservation. While environmental considerations are infrequently observed, their occurrence among specific farmers signifies an increasing awareness of sustainable practices. Furthermore, individuals driven by the collection of varieties (COLM) and the conservation of unique varieties (COUV) typically cultivate specialized orchards that emphasize genetic diversity.

To improve conservation strategies, connecting mango diversity to market opportunities could incentivize economically motivated farmers, while promoting value addition through diversity fairs or roadside stalls may increase financial returns. National policies ought to prioritize the registration of farmers' varieties and the enhancement of skills in grafting and genetic resource management. Promoting community involvement via social incentives can enhance conservation initiatives, and emphasizing environmental advantages may entice additional farmers to embrace sustainable practices. This analysis highlights that custodian farmers are motivated by a variety of economic, personal, social, ancestral, and environmental factors; customized strategies that address these motivations can substantially improve mango diversity conservation while ensuring sustainability and profitability for farmers.

Fig. 2 depicts the comparative importance of various motivational factors influencing custodian farmers in conserving mango varieties. Economic and personal motivations emerged as the primary factors, with 87 farmers (38.33%) citing each as a crucial reason for their conservation efforts. This indicates that financial incentives and personal satisfaction or passion for agriculture are equally important in motivating farmers. Social factors were identified as the second most significant, motivating 44 farmers (19.38%), suggesting that community engagement and peer influence play a role in their commitment, though to a lesser extent.

Only 8 farmers (3.52%) cited environmental motivations, suggesting that ecological concerns are not a predominant factor for the majority of farmers. Likewise, policies and regulations exerted negligible influence, with only one farmer (0.40%) identifying

them as a motivating factor. This indicates that current policies may not adequately align with the interests or priorities of farmers. The findings indicate that economic and personal factors are the primary motivators for mango variety conservation, whereas environmental and policy-related motivations are significantly less impactful. These insights can assist stakeholders in formulating targeted strategies to promote conservation efforts by aligning them with farmers' principal motivations.

The historical importance of orchards, including the two-century-old ones in Bulandshahr, exemplifies the enduring dedication of custodian farmers to safeguarding biodiversity and the cultural heritage linked to mango cultivation (Rajan *et al.*, 2024). This historical context corresponds with the observations made by Sthapit *et al.,* (2013), who emphasized the persistent significance of traditional knowledge in crop conservation initiatives throughout South Asia (Sthapit *et al.*, 2013). Their endeavours, grounded in cultural heritage and indigenous knowledge, not only aid in the preservation of mango diversity but also bolster social and economic resilience within their communities (Rajan *et al.*, 2024). The conservation strategies outlined by Gajanana *et al.,* (2015) emphasize the significant implications of preserving traditional varieties for food security and ecosystem services.

The analysis of motivational factors affecting custodian farmers in mango variety conservation reveals significant insights into the intricate relationship among personal, economic, and social motivations. The dominance of self-interest and the pursuit of a reliable income highlight the essential influence of economic factors in agricultural practices. Considering that mango cultivation frequently serves as a principal source of income for these farmers, it is unsurprising that motivations associated with financial stability are highly prioritized. This indicates that conservation strategies designed to improve mango varieties must also take into account the economic conditions encountered by farmers.

The crucial function of sustainable use (SUSE) indicates a growing consciousness among farmers about the significance of long-term resource management. This motivation signifies a transition towards more accountable agricultural practices, wherein farmers acknowledge that preserving mango varieties can result in sustainable income and ecological well-being. As sustainability gains prominence in global agriculture, initiatives that advocate for optimal practices in mango cultivation may align with farmers' motivations and bolster conservation efforts (Vasudeva *et al.*, 2015).

The emotional ties to mango cultivation, reflected in motivations linked to attachment and affection for mangoes (ATMA) and personal heritage (ANCP), underscore the cultural importance of mangoes in the lives of these farmers. These intrinsic motivations indicate that conservation initiatives should prioritize not only economic incentives but also the celebration and reinforcement of the cultural heritage linked to mango farming. Programs that integrate storytelling, community events, and educational initiatives regarding the historical and cultural significance of mango varieties could enhance emotional connections and promote more effective conservation practices.

Social factors, including social prestige (SOPR) and distribution among friends and relatives (DAFR), suggest that community dynamics influence farmers' motivation. This indicates that promoting community involvement via collaborative endeavours or local organizations may improve conservation initiatives. Utilizing social networks, stakeholders can establish platforms for knowledge exchange, resource aggregation, and collaborative action, thereby enhancing individual initiatives for mango variety conservation.

Notably, external factors such as government assistance (MGOV), export potential (EXOM), market opportunities (MRKT), and environmental issues (ENVN) were determined to exert negligible influence on farmers' motivations. This underscores a possible disparity between policy initiatives and the actual conditions encountered by farmers. It indicates that government programs may not be adequately aligned with the needs and priorities of custodian farmers. To bridge this gap, policymakers must directly engage with farmers to gain a deeper understanding of their perspectives and formulate customized interventions that align with their motivations.

Farmers in Uttar Pradesh cultivate a variety of mango cultivars as a risk management strategy to stabilize income amidst market fluctuations, pest infestations, and climatic variability. The robust correlation (r=0.997) between the quantity of traditional varieties and on-farm conservation success in UP underscores the importance of diversity in facilitating staggered fruit maturity, thereby prolonging market availability and diminishing dependence on monocultures (Rajan *et al.*, 2024). This is consistent with Smale *et al.,* (2004) and Smale (2006), who contend that crop diversity mitigates economic uncertainties by offering multiple sources of income. Nurseries in Malihabad, Lucknow, serve as economic

centres by providing planting materials for traditional varieties and generating livelihood opportunities associated with biodiversity. Kontoleon *et al.,* (2007) observe that market-driven approaches frequently overlook non-commercial varieties, thereby requiring policy interventions to promote conservation via subsidies or acknowledgement of ecosystem services.

Custodian farmers in Uttar Pradesh enjoy social prestige through the conservation of rare varieties, thereby elevating their status as leaders of community knowledge. This reflects findings from Bihar and Jharkhand, where farmers cultivating over ten varieties are esteemed as scion donors and advisors (Singh *et al.*, 2015; 2019). Indigenous Traditional Knowledge (ITK) forms the foundation of conservation practices, including the selection of disease-resistant varieties and grafting techniques, which are transmitted across generations. Subedi *et al.* (2003) assert that these knowledge networks promote collective action, essential for the conservation of agro-biodiversity. Labour shortages and youth migration jeopardize these social structures, as evidenced in Bihar, highlighting the necessity for institutional support to involve younger generations.

The historical and cultural connection to mango diversity is significant. The 250-year-old orchard in Bulandshahr epitomises the custodians' role as heritage protectors, illustrating the documentation of Nawab Hasan's endeavours to preserve heirloom varieties in Kasmandi Kalan, Malihabad (Rajan *et al.*, 2013). Mangoes are vital for local festivals, ceremonies of faith, and culinary traditions, therefore highlighting their cultural importance. Often, cultural identity drives farmers to keep kinds of symbolic significance even though they are less profitable (Sthapit *et al.*, 2013; Rajan *et al.*, 2015). Despite commercial pressures, Saharanpur farmers set aside large orchard areas for traditional mango types, therefore reflecting Negi's (2003) claim that landraces are "living memories" of agricultural legacy.

While UP's custodian farmers show good on-farm preservation, commercial mono-cultures (e.g., Dashehari) pose risks. Pascual and Perrings (2007) suggest combining economic incentives with cultural values, as shown by the certification of traditional types for niche markets. While Swanson (1996) stresses the worldwide public benefit resulting from the preservation of genetic resources, Padulosi (2011) underlines participatory approaches for including custodian farmers in national biodiversity plans. Enhancing Indigenous Traditional Knowledge (ITK) through formal acknowledgement, as suggested by Rajan *et al.,* (2015), may integrate traditional methodologies with scientific inquiry, thereby fortifying resilience against climate change and biotic pressures.

The infrequent occurrence of motivations linked to environmental concerns signifies a necessity for heightened awareness of ecological issues among farmers. Although sustainability is acknowledged as significant, there may be a restricted comprehension of how conservation practices can directly enhance both livelihoods and the environment. Educational initiatives designed to emphasize the ecological advantages of preserving diverse mango varieties may assist in closing this knowledge gap.

Conclusion

The study indicates that intrinsic motivations, including self-interest, income generation, sustainable utilization, and emotional attachment, are the principal catalysts for mango conservation initiatives. Social factors such as prestige and heritage are influential, whereas external influences like government policies or market opportunities exert minimal impact. These insights may help stakeholders design targeted interventions that relate to farmers' main motivations to increase the uptake of conservation practices. This emphasizes the importance of finding a balance between nature conservation, including economic viability, cultural value, community, and nature awareness. By linking conservation strategies to the intrinsic motivations of custodian farmers, stakeholders can develop more efficient and sustainable practices to promote mango conservation while ensuring the sustainability of farmers' livelihoods.

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RESEARCH ARTICLE

Comparative Study of Sapota Varieties and Hybrids for Tolerance and Susceptibility against Seed Borer (*Trymalitis margarias* Meyrick)

K D Bisane[1]*, P K Modi[1] and A P Patel[1]

Abstract

The performance of 23 sapota varieties/hybrids was tested for their ability to withstand seed borer *Trymalitis margarias* Meyrick at Fruit Research Station, Navsari Agricultural University, Gandevi, Gujarat. The varietal deviation for five consecutive years (2015-16 to 2019-20) showed that Kirthibarthi had a higher fruit infestation of 7.47% than the standard check Kalipatti (6.89%), as well as being more sensitive to DHS-2 (6.58%), CO-2 (6.56%), and Cricket balls (5.89%). In contrast, Chala collection-1, Chala collection-3, Chala collection-2, Zumakhiya, CO-3 and CO-1, all exhibited infestation level less than 4%. Fruit damage intensity was highest during the initial phase of fruit harvesting in December, reaching 8.64, 7.94, and 7.67% in Kirthibarthi, Kalipatti, and CO-2, respectively. Nonetheless, DHS-2 (8.54%) and Cricket ball (6.97%) suffered the most damage in February during the middle phase of fruit harvesting. However, a non-significant correlation was revealed between seed morphology *viz.*, length, width and thickness, as well as eight biochemical contents of the seed and pest infestation. Due to late flowering and ultimately fruiting initiation, escape-type resistance was seen in less damaged sapota varieties/hybrids.

Keywords: Hybrids, Sapota, Seed borer, *Trymalitis margarias*, Varieties

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Introduction

Sapota is a crucial tropical fruit in India, with South Gujarat districts of Navsari, Valsad, and Surat accounting for nearly half of the state's sapota production (Anonymous, 2018). Pest interference during the peak flowering and fruiting phase is mainly a concern with the productivity of sapota due to long, asymmetrical and overlapping flowering and fruiting bearing patterns. About 33 insect pests in India and 23 in Gujarat are responsible for significant losses in sapota yield and quality, among different factors (Bisane *et al.,* 2018). Among pests affecting sapota, seed borer, *Trymalitis margarias* Meyrick (Lepidoptera: Tortricidae) has emerged as a serious threat, particularly at the peak fruiting stage. This pest directly damage the seeds, causing deterioration in fruit quality, which affects both domestic sales and export potential. The seed borer is an exotic pest, first detected in India 2000 (Patel, 2001) and now established in major sapota-growing regions, including Gujarat, Maharashtra, Tamil Nadu, Karnataka, and Chhattisgarh (Bisane *et al.,* 2018)**.** The pest caused a significant 40% fruit damage after its initial detection in 2003-04 (AICRP Report, 2004), and continues to cause up to 15% fruit loss in parts of

Gujarat (Bisane, 2016;Bisane and Naik, 2021). Among other regions, fruit loss of 5-10% was noted during December-February in Tamil Nadu (AICRP Report, 2015 and 2016), as well as higher infestation between 30-50% reported from different parts of Karnataka (Kalpana, 2003; Jayanthi and Verghese, 2007 and Patil *et al.,* 2020).

In Gujarat, the Kalipatti variety is preferred by growers for its quality and taste; however, there is a lack of research on how different sapota varieties perform in terms of resistance to seed borer damage. The study aims to fill the knowledge gap regarding varietal resistance/susceptibility to seed borer, which could help mitigate the pest's impact and improve sapota production in affected regions. The findings could guide growers in selecting resistant varieties and contribute to reducing fruit losses due to this pest.

Materials and Methods

The research aims to explore and compare the resistance/susceptibility of various sapota varieties/hybrids to seed borer, *T. margarias* in South Gujarat context was carried out for five consecutive years from 2015-16 to 2019-20 in germplasm plot of ICAR-AICRP (Fruits), Fruit Research Station, Navsari Agricultural University, Gandevi, Gujarat (20.807545° N 73.022260° E). The pest seasonal occurrence based on fruit damage on 23 varieties/hybrids of sapota with many have IC numbers *viz*., PKM-1 (IC-395173), PKM-2 (IC-395184), PKM-3 (IC-395181), PKM-4, PKM-5, CO-1 (IC-395166), CO-2 (IC-395167), CO-3 (IC-395182), DHS-1 (IC-395174), DHS-2 (IC-395183), Murabba (IC-614619), Mohangoottee (IC-395157), Zumakhiya (IC-395159), Bhuripatti, Pilipatti (IC-395155), Cricket ball, (IC-395153) Singapore (IC-395178), Kirthibirthi (IC-395160), Paria Collection, Chala collection-1, Chala collection-2, Chala collection-3 and Kalipatti (IC-395179) was screened on three replicated trees planted at normal 10 m × 10 m spacing. The varieties/hybrids depicting PKM series developed at HC & RI, TNAU, Periyakulam; CO series at TNAU, Coimbatore (Tamil Nadu) and naming DHS developed at UHS, Bagalkot (Karnataka), while others are selected local collections from Navsari and Valsad districts of Gujarat. Kalipatti is considered a standard check to test the differences among them. The trial plot was kept free from any insecticidal spray during the screening phase.

In the sapota orchard, 100 fruits of each variety/hybrid from each tree were selected at every harvesting scheduled from November to April for the incidence of seed borer. The damaged fruits were counted out of 100 fruits to calculate per cent of fruit infestation. The average damage of all six month-wise observations was statistically analyzed in a random block design with three replications (one tree as one replication). Both yearly average and peak damage intensity were considered to decide the tolerance and susceptibility level. Also, the damage difference response of different varieties/hybrids over Kalipatti was calculated to check the susceptibility/ tolerance level.

$$\% \text{ Variation} = \frac{\% \text{ Damage in variety} - \% \text{ Damage in standard check}}{\% \text{ Damage in standard check}} \times 100$$

The fruit damage of different sapota varieties/hybrids was used to categorise the susceptibility/resistance scale against seed borer into six groups. For this purpose, the average per cent fruit damage value of individual varieties/hybrids (Xi) was compared with the grand mean per cent value of all varieties/hybrids (X) and standard deviation (SD) by using the following statistical formula (Gupta and Kapoor, 1997).

Category		Scale formula
Highly Resistant	HR	$Xi \leqslant (X - 2\,SD)$
Resistant	R	$(X - SD) \geqslant Xi > (X - 2\,SD)$
Moderately Resistant	MR	$X \geqslant Xi > (X - SD)$
Moderately Susceptible	MS	$X < Xi \leqslant (X + SD)$
Susceptible	S	$(X + SD) < Xi \leqslant (X + 2\,SD)$
Highly Susceptible	HS	$Xi > (X + 2\,SD)$

For correlation matrix analysis for seed morphology, 10 seeds were sampled randomly from ripened fruits of different varieties/hybrids and measured for length, width, and thickness. Additionally, the standard deviation was computed in order to evaluate the relevance. The eight biochemical contents *viz*., crude protein, total carbohydrate, reducing sugar, non-reducing sugar, total soluble sugar, crude fibre, total phenol, and lipid from seed, were quantified at Food Quality Testing Laboratory, N. M. College of Agriculture, NAU, Navsari to confirm a correlation with fruit damage.

Results

The freshly hatched larvae of seed borer, *T. margarias* build pathways through the fruit pulp and bore holes in

the marble-sized fruit's surface. Infested fruits do not exhibit any outward signs of seed borer damage. The only indication of pest damage is a tiny exit hole, and the mature larvae escape from the infested fruit after finishing feeding on the seed cotyledon (Bisane, 2016). Table 1 to 4 shows the results of five consecutive fruit harvesting seasons' worth of sapota varieties/hybrids evaluation against the seed borer.

During the first year (2015-16), Kirthibarthi (9.23%), CO-2 (8.91%), and DHS-2 (7.26%) had substantially higher annual average fruit damage than Kalipatti (6.72%) and exhibited 8.01–37.29% more susceptibility response (Table 1). However, Chala collection-1, PKM-4 Chala collection-3, and Singapore all found lower mean fruit damage than 4% and had 41.27–58.04% lower vulnerability reactions than Kalipatti. Kirthibarthi (13.56%) and CO-2 (11.94%) had the most fruit damage in November, whereas Kalipatti (10.33%) had the most fruit infestation in December at the beginning of fruit harvesting. In contrast, DHS-2 (12.67%) experienced the most serious damage in February, which was the middle of the fruit harvest.

During the succeeding second year of 2016-17 (Table 1), the higher average fruit damage was recorded in DHS-2 (6.87%), Kirthibarthi (6.12%), Cricket ball (5.69%) than Kalipatti (5.31%) and these varieties/ hybrids had 7.11 to 29.42% more susceptibility response than Kalipatti. However, CO-3 (2.57%), Chala collection-1 (2.60%), CO-1 (2.78%), and Zumakhiya (3.02%) had the minimum mean fruit loss with 43.14-51.64% less susceptibility response than Kalipatti. During April, fruit damage intensity reached its highest in DHS-2 (9.88%), Murabba (8.33%) and DHS-1 (8.08%) at the end of fruit harvesting. While Kirthibarthi (6.67%) had the highest fruit damage in November, and Kalipatti (6.00%) in December at the early fruiting stage.

In the subsequent third year of 2017-18 (Table 2), the yearly mean higher fruit loss was recorded in Kirthbarthi (6.96%) than Kalipatti (6.22%) and also statistically similar to Cricket ball (5.99%), DHS-2 (5.75%) and CO-2 (5.69%). Here, Kirthbarthi was found nearly 11.78% more susceptible than Kalipatti; however, Cricket ball, DHS-2 and CO-2 were only 3.81-8.54% less susceptible. However, the mean lower fruit damage was recorded in Chala collection-3 (2.41%), Chala collection-1 (2.45%), PKM-3 (2.71%), CO-3 (2.75%) and CO-1 (2.82%), which showed 54.74 to 61.27% less receptiveness reaction over Kalipatti. Unlike the earlier two seasons, the fruit damage reached its highest in December only during 2017-18 in susceptible varieties/hybrids *viz.*, Kirthbarthi (8.33%), Kalipatti (7.00%), CO-2 (6.57%), Cricket ball (6.56%) and DHS-2 (6.33%) at the initial phase of fruit harvesting.

In 2018-19 (IV year), seed borer caused higher fruit damage in Kalipatti (8.39%), after that in Kirthibharti (7.42%), DHS-2 (6.53%), CO-2 (6.16%) and Paria collection (6.03%) and were only 11.57-28.15% less susceptible than Kalipatti (Table 2). While the lower damage of marketable fruits was observed in Chala collection-2 (1.63%), Chala collection-3 (2.09%), CO-3 (2.26%) and Chala collection-1 (2.38%) and showed 71.65 to 80.53% less receptiveness reaction over Kalipatti. The maximum infestation was observed during December in Kalipatti (9.44%), Kirthibharti (9.15%), CO-2 (8.52%) and Paria collection (7.33%), as well as during February in DHS-2 (8.43%).

In the fifth year (2019-20), the average fruit damage was higher in CO-2 (7.88%), closely by Kalipatti (7.80%), Kirthibharti (7.64%), Cricket Ball (6.57%), DHS-2 (6.50%), and Paria collection (6.40%) (Table 3). Among them, CO-2 was found to be 0.97% more susceptible than Kalipatti; however, Kirthibharti, Cricket Ball, DHS-2, and Paria collection exhibited only 2.05-18.01% less susceptibility, performing similarly to the standard check. In contrast, the lower damage was observed in Chala Collection-2 (2.08%), Chala Collection-1 (2.25%), Chala Collection-3 (2.34%), Zumakhiya (2.65%), and Singapore (2.78%) and demonstrated significantly lower receptiveness of 64.42-73.35% than Kalipatti. Peak infestation was noted in February, particularly in Kirthibharti (10.68%), CO-2 (10.37%) and Cricket Ball (9.52%). Similarly, in March, Kalipatti (10.33%) and DHS-2 (8.58%) showed significant damage during the advanced fruiting phase.

In pooled data spanning five years (Table 3), Kirthibarthi had the highest average seed borer infestation (7.47%), followed by Kalipatti (6.89%). The varieties DHS-2 (6.58%), CO-2 (6.56%), Cricket ball (5.89%), and Paria collection (5.35%) also showed relatively high infestation, indicating their susceptibility to seed borer. Here, Kirthibarthi exhibited up to 8.48% higher susceptibility to seed borer compared to Kalipatti and closer to DHS-2 and CO-2, which showed decreased vulnerability with 4.45-4.77% lesser infestation rates compared to the standard check. Meanwhile, the Cricket ball and Paria collection were 14.53-22.34% less susceptible than Kalipatti. In contrast, varieties Chala collection-1 (2.50%), Chala collection-3 (2.75%), Chala collection-2 (2.94%), Zumakhiya (3.21%), CO-3 (3.24%), and CO-1 (3.30%) experienced significantly lower infestation levels, demonstrating better resilience to seed borer with overall 52.11 to 63.70% less susceptibility response than Kalipatti.

Table 1: Performance of different varieties/hybrids of sapota against seed borer (I and II year)

Treat-ment	Varieties/ hybrids	(I year- 2015-16)					(II year- 2016-17)				
		Avg. % fruit damage*	Max. fruit damage (%)	Max. activity month	Min. fruit damage (%)	Per cent variation over Kalipatti	Avg. % fruit damage*	Max. fruit damage (%)	Max. activity month	Min. fruit damage (%)	Per cent variation over Kalipatti
T_1	PKM-1	4.80 (2.28)	6.33	December	3.33	-28.56	4.72 (2.26)	7.08	April	3.24	-11.05
T_2	PKM-2	4.37 (2.17)	5.68	November	2.50	-35.05	3.79 (2.06)	5.22	January	2.80	-28.59
T_3	PKM-3	4.14 (2.13)	6.39	November	2.61	-38.43	3.95 (2.05)	7.66	April	2.44	-25.61
T_4	PKM-4	3.50 (1.98)	4.57	November	2.80	-47.97	3.74 (2.00)	7.90	April	1.89	-29.53
T_5	PKM-5	4.43 (2.21)	5.87	November	3.73	-34.07	3.58 (1.99)	5.85	April	2.30	-32.54
T_6	CO-1	4.61 (2.20)	7.00	December	2.53	-31.40	2.78 (1.80)	3.19	December	2.33	-47.70
T_7	CO-2	8.91 (3.00)	11.94	November	3.11	32.51	4.16 (2.14)	5.46	November	2.50	-21.60
T_8	CO-3	4.91 (2.26)	7.67	December	2.78	-26.90	2.57 (1.73)	3.17	December	1.98	-51.64
T_9	DHS-1	4.44 (2.19)	5.64	November	2.17	-33.92	3.84 (2.02)	8.08	April	2.05	-27.59
T_{10}	DHS-2	7.26 (2.71)	12.67	February	3.33	8.01	6.87 (2.69)	9.88	April	4.00	29.42
T_{11}	Murabba	4.50 (2.19)	7.33	December	2.50	-33.04	4.88 (2.27)	8.33	April	2.17	-8.01
T_{12}	Mohangootee	4.74 (2.24)	7.67	November	2.90	-29.41	4.03 (2.07)	6.63	April	1.70	-24.10
T_{13}	Zumakhiya	4.05 (2.09)	5.67	April	2.24	-39.76	3.02 (1.86)	4.33	April	2.17	-43.14
T_{14}	Bhuripatti	4.06 (2.09)	6.33	December	2.07	-39.57	3.64 (2.02)	4.84	December	2.78	-31.34
T_{15}	Pilipatti	4.68 (2.24)	7.10	December	2.55	-30.38	3.25 (1.92)	4.35	January	2.24	-38.79
T_{16}	Cricket ball	5.99 (2.48)	8.61	December	2.61	-10.88	5.69 (2.47)	6.06	December	5.22	7.11
T_{17}	Singapore	3.95 (2.07)	4.89	April	2.19	-41.27	3.83 (2.04)	5.42	April	1.67	-27.81
T_{18}	Kirthibarthi	9.23 (3.05)	13.56	November	4.67	37.29	6.12 (2.56)	6.67	November	5.00	15.27
T_{19}	Paria coll.	4.61 (2.21)	6.92	November	1.96	-31.48	4.90 (2.30)	6.52	April	3.11	-7.76
T_{20}	Chala coll.-1	2.82 (1.80)	3.17	December	2.22	-58.04	2.60 (1.74)	3.98	December	1.90	-51.01
T_{21}	Chala coll.-2	4.26 (2.14)	5.60	December	2.45	-36.61	3.53 (1.99)	4.60	February	2.62	-33.44
T_{22}	Chala coll.-3	3.62 (1.99)	4.80	April	2.74	-46.16	3.30 (1.93)	5.05	April	2.21	-37.75
T_{23}	Kalipatti	6.72 (2.64)	10.33	December	3.00	--	5.31 (2.41)	6.00	December	4.33	--
CD	T	0.21	--	--	--	--	0.16	--	--	--	--
at	M	0.11	--	--	--	--	0.08	--	--	--	--
5%	TxM	0.51	--	--	--	--	0.39	--	--	---	-
CV%		13.90	--	--	--	--	11.47	--	--	--	--

* Figures in parenthesis are (x+0.5) square root transformed values. T = Treatments, M = Month.

Table 2: Performance of different varieties/hybrids of sapota against seed borer (III and IV year)

Treat-ment	Varieties/ hybrids	(III year- 2017-18)					(IV year- 2018-19)				
		Avg. % fruit damage*	Max. fruit damage (%)	Max. activity month	Min. fruit damage (%)	Per cent variation over Kalipatti	Avg. % fruit damage*	Max. fruit damage (%)	Max. activity month	Min. fruit damage (%)	Per cent variation over Kalipatti
T_1	PKM-1	3.62 (2.00)	5.11	April	2.25	-41.90	4.12 (2.13)	5.14	April	2.73	-50.84
T_2	PKM-2	3.21 (1.90)	4.67	April	1.83	-48.47	3.46 (1.97)	4.68	March	2.50	-58.68
T_3	PKM-3	2.71 (1.77)	4.19	April	2.11	-56.41	2.85 (1.80)	3.93	April	1.98	-66.07
T_4	PKM-4	3.25 (1.86)	5.21	April	1.57	-47.80	3.05 (1.85)	3.67	April	1.94	-63.65
T_5	PKM-5	3.43 (1.90)	5.31	April	1.59	-44.89	2.87 (1.74)	4.82	March	0.95	-65.76
T_6	CO-1	2.82 (1.79)	3.83	December	1.85	-54.74	2.65 (1.72)	3.93	December	1.94	-68.43
T_7	CO-2	5.69 (2.47)	6.57	December	4.39	-8.54	6.16 (2.56)	8.52	December	5.24	-26.52
T_8	CO-3	2.75 (1.72)	4.46	March	1.31	-55.83	2.26 (1.59)	3.38	December	1.08	-73.09
T_9	DHS-1	3.56 (1.99)	4.58	April	2.58	-42.87	3.86 (2.06)	5.23	February	2.34	-54.00
T_{10}	DHS-2	5.75 (2.49)	6.33	December	4.67	-7.59	6.53 (2.63)	8.43	February	0.00	-22.16
T_{11}	Murabba	4.16 (2.14)	5.22	December	2.50	-33.09	4.37 (2.18)	6.27	February	2.69	-47.88
T_{12}	Mohangootee	4.37 (2.18)	6.22	December	3.00	-29.79	4.14 (2.13)	4.90	February	2.98	-50.67
T_{13}	Zumakhiya	3.63 (2.01)	4.22	March	2.93	-41.62	2.72 (1.76)	3.43	March	1.47	-67.57
T_{14}	Bhuripatti	4.10 (2.13)	5.56	December	3.25	-34.07	3.53 (1.99)	4.45	December	2.33	-57.96
T_{15}	Pilipatti	4.11 (2.14)	5.00	December	3.31	-33.96	3.83 (2.05)	5.26	December	3.07	-54.37
T_{16}	Cricket ball	5.99 (2.54)	6.56	December	5.33	-3.81	5.21 (2.30)	6.41	December	4.55	-37.92
T_{17}	Singapore	4.19 (2.15)	5.67	April	2.83	-32.69	3.77 (2.04)	4.48	March	2.52	-55.10
T_{18}	Kirthibarthi	6.96 (2.72)	8.33	December	5.97	11.78	7.42 (2.80)	9.15	December	6.45	-11.57
T_{19}	Paria coll.	4.82 (2.29)	6.22	December	3.67	-22.51	6.03 (2.54)	7.33	December	5.14	-28.15
T_{20}	Chala coll.-1	2.45 (1.67)	3.69	December	1.37	-60.63	2.38 (1.67)	3.55	March	1.56	-71.65
T_{21}	Chala coll.-2	3.20 (1.84)	4.81	December	2.17	-48.66	1.63 (1.36)	2.27	March	0.61	-80.53
T_{22}	Chala coll.-3	2.41 (1.61)	4.44	April	1.43	-61.27	2.09 (1.48)	3.56	March	1.03	-75.05
T_{23}	Kalipatti	6.22 (2.58)	7.00	December	5.33	--	8.39 (2.96)	9.44	December	6.56	--
CD	T	0.22	--	--	--	--	0.26	--	--	--	--
at	M	0.11	--	--	--	--	0.13	--	--	--	--
5%	TxM	NS	--	--	--	--	NS	--	--	--	--
	CV%	16.21	--	--	--	--	19.16	--	--	--	--

* Figures in parenthesis are (x+0.5) square root transformed values. T = Treatments, M = Month.

Table 3: Performance of different varieties/hybrids of sapota against seed borer (V year and Pooled)

Treat-ment	Varieties/ hybrids	(V year- 2019-20)					5 years pooled				
		Avg. % fruit damage*	Max. fruit damage (%)	Max. activity month	Min. fruit damage (%)	Per cent variation over Kalipatti	Avg. % fruit damage*	Max. fruit damage (%)	Max. activity month	Min. fruit damage (%)	Per cent variation over Kalipatti
T_1	PKM-1	3.80 (2.03)	5.71	March	1.45	-51.32	4.21 (2.14)	5.14	April	3.31	-38.85
T_2	PKM-2	3.07 (1.83)	4.64	April	1.14	-60.71	3.58 (1.99)	4.36	March	3.01	-48.05
T_3	PKM-3	4.65 (2.21)	8.59	April	2.38	-40.45	3.66 (1.99)	5.53	April	2.72	-46.89
T_4	PKM-4	3.64 (2.02)	4.75	February	2.33	-53.29	3.44 (1.94)	4.74	April	2.46	-50.12
T_5	PKM-5	4.58 (2.23)	6.33	April	3.38	-41.36	3.78 (2.01)	5.09	April	2.82	-45.16
T_6	CO-1	3.64 (2.01)	4.92	February	2.33	-53.33	3.30 (1.90)	4.38	December	2.59	-52.11
T_7	CO-2	7.88 (2.86)	10.37	February	4.03	0.97	6.56 (2.61)	7.67	December	5.83	-4.77
T_8	CO-3	3.71 (2.02)	5.02	April	2.53	-52.44	3.24 (1.87)	3.78	December	2.62	-52.97
T_9	DHS-1	3.99 (2.09)	4.92	February	2.37	-48.92	3.94 (2.07)	4.92	April	2.68	-42.85
T_{10}	DHS-2	6.50 (2.60)	8.58	March	3.06	-16.69	6.58 (2.62)	8.54	February	5.38	-4.45
T_{11}	Murabba	3.29 (1.90)	5.24	February	1.39	-57.83	4.24 (2.13)	4.94	December	3.19	-38.42
T_{12}	Mohangootee	2.95 (1.83)	4.33	February	1.45	-62.15	4.05 (2.09)	4.85	December	2.98	-41.25
T_{13}	Zumakhiya	2.65 (1.70)	4.42	February	0.67	-66.04	3.21 (1.88)	3.99	April	2.62	-53.34
T_{14}	Bhuripatti	3.51 (1.96)	4.58	February	2.22	-55.04	3.77 (2.04)	4.74	December	3.27	-45.29
T_{15}	Pilipatti	3.68 (1.98)	5.24	April	1.22	-52.85	3.91 (2.07)	4.42	December	3.44	-43.25
T_{16}	Cricket ball	6.57 (2.62)	9.52	February	3.79	-15.82	5.89 (2.48)	6.97	February	5.08	-14.53
T_{17}	Singapore	2.78 (1.76)	4.43	February	1.22	-64.42	3.70 (2.01)	4.60	April	2.67	-46.26
T_{18}	Kirthibarthi	7.64 (2.81)	10.68	February	3.79	-2.05	7.47 (2.79)	8.64	December	6.35	8.48
T_{19}	Paria coll.	6.40 (2.54)	8.59	April	2.06	-18.01	5.35 (2.38)	6.02	December	4.45	-22.34
T_{20}	Chala coll.-1	2.25 (1.64)	2.79	February	1.86	-71.13	2.50 (1.7)	2.97	December	2.26	-63.70
T_{21}	Chala coll.-2	2.08 (1.48)	3.25	February	1.31	-73.35	2.94 (1.76)	3.47	February	2.40	-57.32
T_{22}	Chala coll.-3	2.34 (1.56)	3.02	February	1.79	-69.99	2.75 (1.72)	3.65	April	2.22	-60.02
T_{23}	Kalipatti	7.80 (2.83)	10.33	March	3.31	--	6.89 (2.69)	7.94	December	6.04	--
CD	T	0.26	--	--	--	--	0.10	--	--	--	--
at	M	0.13	--	--	--	--	0.05	--	--	--	--
5%	Y	--	--	--	--	--	0.05	--	--	--	--
	TxM	NS	--	--	--	--	0.25	--	--	--	--
	TxY	--	--	--	--	--	0.22	--	--	--	--
	MxY	--	--	--	--	--	0.11	--	--	--	--
	TxMxY	--	--	--	--	--	NS	--	--	--	--
CV%		18.59	--	--	--	--	16.11	--	--	--	--

* Figures in parenthesis are (x+0.5) square root transformed values. T = Treatments, M = Month.

December had the highest fruit damage intensity during the initial phase of fruit harvesting. Damage levels peaked in Kirthibarthi (8.64%), Kalipatti (7.94%), CO-2 (7.67%), and Paria collection (6.02%) (Table 4).

For DHS-2 (8.54%) and Cricket ball (6.97%), peak damage was recorded in February, aligning with the middle of the harvest season. The higher infestation due to seed borer was recorded mostly in December and a few in April among all varieties/hybrids (Table 5). In December, a maximum of 11 varieties/hybrids were

Table 4: Month-wise performance of different varieties/hybrids of sapota against seed borer (5 years pooled)

Treatment	Varieties/ hybrids	Per cent Fruit damage (5 years pooled)					
		Nov.	Dec.	Jan.	Feb.	March	April
T_1	PKM-1	3.31 (1.90)	3.70 (2.01)	3.95 (2.09)	4.18 (2.15)	4.99 (2.33)	5.14 (2.35)
T_2	PKM-2	3.02 (1.82)	3.01 (1.84)	3.33 (1.93)	3.94 (2.08)	4.36 (2.19)	3.81 (2.05)
T_3	PKM-3	3.28 (1.90)	2.95 (1.84)	2.72 (1.77)	3.30 (1.92)	4.17 (2.13)	5.53 (2.39)
T_4	PKM-4	2.46 (1.65)	3.06 (1.87)	3.01 (1.84)	3.42 (1.97)	3.92 (2.08)	4.74 (2.23)
T_5	PKM-5	2.82 (1.71)	3.32 (1.89)	3.39 (1.92)	3.71 (2.03)	4.34 (2.18)	5.09 (2.34)
T_6	CO-1	3.11 (1.84)	4.38 (2.17)	3.67 (1.98)	3.19 (1.90)	2.59 (1.72)	2.85 (1.80)
T_7	CO-2	6.43 (2.58)	7.67 (2.82)	6.49 (2.61)	6.80 (2.61)	6.15 (2.55)	5.83 (2.48)
T_8	CO-3	3.01 (1.78)	3.78 (1.99)	3.39 (1.90)	3.15 (1.89)	3.49 (1.96)	2.62 (1.69)
T_9	DHS-1	3.36 (1.93)	4.00 (2.09)	2.68 (1.76)	4.00 (2.09)	4.66 (2.26)	4.92 (2.30)
T_{10}	DHS-2	5.66 (2.44)	6.74 (2.67)	5.38 (2.39)	8.54 (2.98)	6.67 (2.65)	6.50 (2.60)
T_{11}	Murabba	3.89 (2.03)	4.94 (2.29)	3.19 (1.89)	4.09 (2.11)	4.92 (2.32)	4.42 (2.17)
T_{12}	Mohangootee	4.39 (2.14)	4.85 (2.27)	2.98 (1.84)	3.43 (1.95)	4.40 (2.2)	4.23 (2.13)
T_{13}	Zumakhiya	2.62 (1.67)	3.43 (1.93)	2.69 (1.77)	2.96 (1.84)	3.60 (2.01)	3.99 (2.09)
T_{14}	Bhuripatti	3.55 (1.96)	4.74 (2.26)	3.57 (2.01)	3.27 (1.92)	3.61 (2.00)	3.88 (2.07)
T_{15}	Pilipatti	3.62 (1.97)	4.42 (2.17)	4.16 (2.14)	3.53 (1.99)	3.44 (1.97)	4.28 (2.16)
T_{16}	Cricket ball	5.40 (2.40)	6.88 (2.64)	5.50 (2.39)	6.97 (2.71)	5.08 (2.34)	5.49 (2.42)
T_{17}	Singapore	3.32 (1.90)	4.13 (2.11)	2.67 (1.76)	3.57 (1.99)	3.92 (2.09)	4.60 (2.23)
T_{18}	Kirthibarthi	7.39 (2.74)	8.64 (3.00)	7.86 (2.85)	7.50 (2.81)	7.10 (2.73)	6.35 (2.60)
T_{19}	Paria coll.	4.71 (2.23)	6.02 (2.53)	4.45 (2.19)	5.70 (2.46)	5.26 (2.34)	5.95 (2.51)
T_{20}	Chala coll.-1	2.26 (1.63)	2.97 (1.84)	2.53 (1.72)	2.27 (1.64)	2.41 (1.66)	2.56 (1.74)
T_{21}	Chala coll.-2	2.99 (1.76)	3.12 (1.79)	2.40 (1.62)	3.47 (1.89)	2.71 (1.73)	2.94 (1.79)
T_{22}	Chala coll.-3	2.22 (1.55)	2.65 (1.69)	2.38 (1.62)	2.37 (1.63)	3.26 (1.87)	3.65 (1.95)
T_{23}	Kalipatti	6.04 (2.53)	7.94 (2.89)	6.40 (2.61)	7.13 (2.73)	6.80 (2.65)	7.01 (2.71)
Avg. % fruit damage of the month		3.86	4.67	3.86	4.37	4.43	4.63
CD at 5%	T	0.26	0.25	0.26	0.23	0.24	0.24
	M	0.12	0.12	0.12	0.10	0.11	0.11
	TxM	0.58	0.57	0.58	0.50	0.53	0.54
CV%		17.92	15.97	17.66	14.61	15.15	15.26

* Figures in parenthesis are (x+0.5) square root transformed values. T = Treatments, M = Month.

Table 5: Month-wise trend of seed borer fruit damage in different varieties/hybrids of sapota

Year/Months	Nov.	Dec.	Jan.	Feb.	March	April
2015-16	9	10	-	1	-	3
2016-17	2	6	2	1	-	12
2017-18	-	13	-	-	2	8
2018-19	-	9	-	4	7	3
2019-20	-	-	-	14	3	6
Avg.	-	11	-	3	1	8

Note: Figures in tables indicate no. of varieties/hybrids with peak damage.

infested by seed borer, as well as 8 in April. Other months have less damage intensity. This type of trend was also noted in year-wise screening data, except for 2019-20.

Compared to previous years, a wide variety experienced peak fruit damage later, in March-April, due to a delayed onset of the fruiting season. In month-wise and year-wise fruit damage over the five years, there were significant variations noted, as well as in the mean values across six months of observations. As well, no significant interaction effects were detected when comparing data from 2017-18 to 2019-20. However, the interaction outcome was significant during 2015-16 and 2016-17. The reason for this mismatch could be that the first two years had a higher infestation of seed borer with substantial variation across varieties/hybrids, but the succeeding three years had a decrease in pest incidence, resulting in less dissimilarity.

The outcome of categorization of different varieties/hybrids of sapota based on mean fruit damage by seed borer is depicted in Table 6. In pooled grouping, none of the varieties/hybrids were found highly resistant; however, Chala collection-1 and 3 were ranked as resistant, while 15 varieties/ hybrids were grouped as moderately susceptible. Paria collection was enlisted under the moderately susceptible category, whereas CO-2, DHS-2, Cricket ball and standard check- Kalipatti were enlisted in the susceptible and Kirthibarthi under the highly susceptible category. Like, the scaling classification

Table 6: Categorization of different varieties/hybrids of sapota based on average fruit damage due to seed borer

Scale	2015-16	2016-17	2017-18	2018-19	2019-20	Pooled
Highly Resistant (HR)	--	--	--	--	--	--
Resistant (R)	Chala coll.-1	CO-1, CO-3, Chala coll.-1	PKM-3, CO-3, Chala coll.-1, Chala coll.-3	CO-3, Chala coll.-2 Chala coll.-3	Chala coll.-1, Chala coll.-2, Chala coll.-3	Chala coll.-1, Chala coll.-3
Moderately Resistant (MR)	PKM-1, PKM-2, PKM-3, PKM-4, PKM-5, CO-1, CO-3, DHS-1, Murabba, Mohangootee, Zumakhiya, Bhuripatti, Pilipatti, Singapore, Paria coll., Chala coll.-2, Chala coll. 3	PKM-2, PKM-3, PKM-4, PKM-5, DHS-1, Mohangootee, Zumakhiya, Bhuripatti, Pilipatti, Singapore, Chala coll.-2, Chala coll.-3	PKM-1, PKM-2, PKM-4, PKM-5, CO-1, DHS-1, Zumakhiya, Chala coll. 2	PKM-2, PKM-3, PKM-4, PKM-5, CO-1, DHS-1, Zumakhiya, Bhuripatti, Pilipatti, Singapore, Chala coll.-1	PKM-1, PKM-2, PKM-4, CO-1, CO-3, DHS-1, Murabba, Mohangootee, Zumakhiya, Bhuripatti, Pilipatti, Singapore	PKM-1, PKM-2, PKM-3, PKM-4, PKM-5, CO-1, CO-3, DHS-1, Murabba, Mohangootee, Zumakhiya, Bhuripatti, Pilipatti, Singapore, Chala coll.-2
Moderately Susceptible (HS)	Cricket ball	PKM-1, CO-2, Murabba, Paria coll.	Murabba, Mohangootee, Bhuripatti, Pilipatti, Singapore, Paria coll.	PKM-1, Murabba, Mohangootee, Cricket ball	PKM-3, PKM-5	Paria coll.
Susceptible (S)	DHS-2, Kalipatti	Cricket ball, Kirthibarthi, Kalipatti	CO-2, DHS-2, Cricket ball, Kalipatti	CO-2, DHS-2, Kirthibarthi, Paria coll.	CO-2, DHS-2, Cricket ball, Kirthibarthi, Paria coll., Kalipatti	CO-2, DHS-2, Cricket ball, Kalipatti
Highly Susceptible(HS)	CO-2, Kirthibarthi	DHS-2	Kirthibarthi	Kalipatti	--	Kirthibarthi

with respect to peak fruit damage is presented in Table 7. Here in pooled data, none of the varieties/hybrids were found highly resistant, while Chala collection-1, 2 and 3 were grouped under the resistant category, and 13 varieties/hybrids were categorised in moderately resistant and PKM-3 and Paria collection in moderately susceptible. CO-2, Cricket ball and Kalipatti were classified as susceptible varieties while DHS-2 and Kirthibarthi come under the highly susceptible category. Under individual years cataloguing under average and peak damage, nearly the same trend was seen in the ranking, and a slight disparity may be due to the crop phenology impact.

The data on bud morphology (Table 8) and eight biochemical contents (Table 9) were measured, and a correlation matrix was assessed with seed damage for

Table 7: Categorization of different varieties/hybrids of sapota based on peak fruit damage due to seed borer

Scale	2015-16	2016-17	2017-18	2018-19	2019-20	Pooled
Highly Resistant (HR)	--	--	--	--	--	--
Resistant (R)	Chala coll.-1	CO-1, CO-3, Chala coll.-1	PKM-3, CO-1, Zumakhiya, Chala coll.-1	Chala coll.-2	Chala coll.-1, Chala coll.-2, Chala coll.-3	Chala coll.-1, Chala coll.-2, Chala coll.-3
Moderately Resistant (MR)	PKM-1, PKM-2, PKM-3, PKM-4, PKM-5, CO-1, DHS-1, Zumakhiya, Bhuripatti, Pilipatti, Singapore, Paria coll., Chala coll.-2, Chala coll.-3	PKM-2, PKM-5, CO-2, Zumakhiya, Bhuripatti, Pilipatti, Singapore, Chala coll.-2, Chala coll.-3	PKM-1, PKM-2, PKM-4, PKM-5, CO-3, DHS-1, Murabba, Pilipatti, Chala coll.-2, Chala coll.-3	PKM-1, PKM-2, PKM-3, PKM-4, PKM-5, CO-1, CO-3, DHS-1, Mohangootee, Zumakhiya, Bhuripatti, Pilipatti, Singapore, Chala coll.-1, Chala coll.-3	PKM-1, PKM-2, PKM-4, CO-1, CO-3, DHS-1, Murabba, Mohangootee, Zumakhiya, Bhuripatti, Pilipatti, Singapore	PKM-1, PKM-2, PKM-4, PKM-5, CO-1, CO-3, DHS-1, Murabba, Mohangootee, Zumakhiya, Bhuripatti, Pilipatti, Singapore
Moderately Susceptible (HS)	CO-3, Murabba, Mohangootee, Cricket ball	PKM-1, Mohangootee, Cricket ball, Kirthibarthi, Paria coll., Kalipatti	DHS-2, Mohangootee, Bhuripatti, Singapore, Paria coll.	Murabba, Cricket ball	PKM-5, DHS-2, Paria coll.	PKM-3, Paria coll.
Susceptible (S)	CO-2, Kalipatti	PKM-3, PKM-4, DHS-1, Murabba	CO-2, Cricket ball, Kalipatti	CO-2, DHS-2, Kirthibarthi, Paria coll.	PKM-3, CO-2, Cricket ball, Kirthibarthi, Kalipatti	CO-2, Cricket ball, Kalipatti
Highly Susceptible (HS)	DHS-2, Kirthibarthi	DHS-2	Kirthibarthi	Kalipatti	--	DHS-2, Kirthibarthi

Table 8: Morphological parameters of seed in different varieties/hybrids of sapota

Treatment	Varieties/ hybrids	Avg. seed length (mm $\pm$ SD)	Avg. seed width (mm $\pm$ SD)	Avg. seed thickness (mm $\pm$ SD)
T_1	PKM-1	19.94 $\pm$ 0.85	10.63 $\pm$ 0.38	5.71 $\pm$ 0.63
T_2	PKM-2	20.60 $\pm$ 0.88	11.99 $\pm$ 0.89	5.80 $\pm$ 0.47

T_3	PKM-3	19.11 ± 3.09	11.74 ± 0.5	6.02 ± 0.74
T_4	PKM-4	21.27 ± 1.33	10.34 ± 0.88	5.86 ± 0.63
T_5	PKM-5	17.05 ± 1.11	8.84 ± 0.74	4.63 ± 0.35
T_6	CO-1	21.34 ± 1.35	12.04 ± 0.68	6.17 ± 0.44
T_7	CO-2	18.55 ± 2.78	9.98 ± 00.64	4.79 ± 0.32
T_8	CO-3	19.26 ± 1.17	11.81 ± 0.67	6.05 ± 0.48
T_9	DHS-1	20.54 ± 0.90	11.14 ± 0.86	5.77 ± 0.44
T_{10}	DHS-2	22.81 ± 0.78	11.34 ± 0.53	5.80 ± 0.27
T_{11}	Murabba	19.94 ± 1.56	11.05 ± 0.81	5.64 ± 1.02
T_{12}	Mohangootee	19.92 ± 1.26	10.14 ± 0.90	5.40 ± 0.40
T_{13}	Zumakhiya	20.12 ± 1.73	10.73 ± 0.94	5.97 ± 0.87
T_{14}	Bhuripatti	20.99 ± 1.19	10.59 ± 0.69	5.32 ± 0.34
T_{15}	Pilipatti	20.89 ± 0.76	11.24 ± 0.94	5.56 ± 0.61
T_{16}	Cricket ball	19.45 ± 0.95	10.31 ± 0.96	5.82 ± 0.58
T_{17}	Singapore	21.59 ± 0.73	11.61 ± 0.53	6.34 ± 0.58
T_{18}	Kirthibarthi	19.50 ± 0.60	9.83 ± 0.36	5.14 ± 0.42
T_{19}	Paria coll.	19.34 ± 1.09	10.65 ± 0.58	5.42 ± 0.31
T_{20}	Chala coll.-1	20.88 ± 0.93	11.63 ± 0.24	5.23 ± 0.40
T_{21}	Chala coll.-2	20.62 ± 1.06	10.91 ± 0.58	5.59 ± 0.60
T_{22}	Chala coll.-3	19.98 ± 1.15	10.71 ± 0.77	5.43 ± 0.35
T_{23}	Kalipatti	22.06 ± 1.11	11.13 ± 1.56	5.73 ± 0.47

Table 9: Biochemical parameters of seed in different varieties/hybrids of sapota

Treatment	Varieties/ hybrids	Crude Protein (%)	Total Carbohydrate (%)	Reducing Sugar (%)	Non-reducing Sugar (%)	Total Soluble Sugar (%)	Crude Fibre (%)	Total Phenol (%)	Lipid (%)
T_1	PKM-1	1.06	74.76	1.35	7.02	8.36	13.00	0.21	11.45
T_2	PKM-2	1.60	75.01	0.14	7.63	7.77	15.12	0.12	10.80
T_3	PKM-3	0.68	78.55	0.48	8.17	8.66	11.20	0.21	14.85
T_4	PKM-4	1.43	65.27	0.78	6.48	7.27	19.56	0.16	8.40
T_5	PKM-5	0.93	86.25	0.06	6.23	6.29	12.76	0.15	14.00
T_6	CO-1	1.72	77.46	0.68	7.72	8.41	13.66	0.32	11.70
T_7	CO-2	1.65	82.40	0.52	6.23	6.75	13.96	0.18	12.30
T_8	CO-3	0.75	77.36	0.43	10.53	10.96	14.96	0.14	13.45
T_9	DHS-1	0.83	89.77	0.08	7.43	7.51	13.23	0.13	13.05
T_{10}	DHS-2	0.48	94.86	0.09	6.85	6.94	14.41	0.13	11.10
T_{11}	Murabba	0.59	73.76	0.20	4.17	4.36	21.16	0.11	10.95
T_{12}	Mohangootee	0.71	69.37	0.29	5.64	5.93	13.92	0.17	11.10
T_{13}	Zumakhiya	2.70	85.45	0.47	6.52	6.99	14.60	0.11	36.40
T_{14}	Bhuripatti	0.58	41.35	0.33	6.47	6.80	15.20	0.11	13.20
T_{15}	Pilipatti	0.83	63.82	0.76	9.33	10.09	11.84	0.27	14.15
T_{16}	Cricket ball	2.04	69.27	0.26	7.07	7.33	16.88	0.14	13.90
T_{17}	Singapore	1.73	65.77	0.29	6.15	6.43	19.04	0.19	8.15
T_{18}	Kirthibarthi	0.99	64.17	0.32	7.08	7.40	14.22	0.20	13.55
T_{19}	Paria coll.	3.91	99.48	0.67	13.27	13.94	13.12	0.17	14.05
T_{20}	Chala coll.-1	0.68	79.55	0.15	8.29	8.45	12.90	0.12	12.60
T_{21}	Chala coll.-2	0.83	78.40	0.77	5.88	6.65	12.12	0.26	14.35
T_{22}	Chala coll.-3	0.87	64.82	2.76	11.06	13.82	15.50	0.32	15.10
T_{23}	Kalipatti	2.48	63.62	0.20	6.94	7.14	12.78	0.20	13.70

their significance. There was a non-significant correlation between seed length, width and thickness of different varieties/hybrids and seed damage with respect to average and peak infestation (Table 10). As well, a non-significant correlation was observed between seed borer damage and eight biochemical contents of seed *viz.*, crude protein, total carbohydrate, reducing sugar, non-reducing sugar, total soluble sugar, crude fibre, total phenol and lipid of different varieties/hybrids (Table 11). This indicates pest population may be influenced by ecological and phenological factors, as well as escape type resistance can be catalogued in less damaged sapota varieties/hybrids due to late flowering and subsequently fruiting initiation.

Discussion

In the past 20 years, relatively little study has been done on the screening of sapota varieties against seed borer throughout India since its detection in 2000; limited information exists, which has been conducted primarily in Gujarat. In earlier studies in South Gujarat, Kanade (2005) revealed that among the different varieties of

Table 10: Correlation matrix of seed morphology parameters of different varieties/hybrids of sapota with fruit damage by seed borer

Parameters	Fruit damage	Seed Length	Seed Width	Seed Thickness
Fruit damage	1	--	--	--
Seed Length	0.021 (0.030)#	1	--	--
Seed Width	-0.332 (-0.324)#	0.581**	1	--
Seed Thickness	-0.254 (-0.212)#	0.551**	0.739***	1

**Correlation is significant at 1% and * Correlation is significant at 5%.
Figures in parenthesis are correlation matrix analyzed for peak fruit damage and outside are correlation matrix analyzed for average fruit damage.

Table 11: Correlation matrix of biochemical parameters of different varieties/hybrids of sapota with fruit damage by seed borer

Parameter	Fruit damage	Crude Protein	Total Carbohydrate	Reducing Sugar	Non-reducing Sugar	Total Soluble Sugar	Crude Fiber	Total Phenol	Lipid
Fruit damage	1	--	--	--	--	--	--	--	--
Crude Protein	0.263 (0.196)#	1	--	--	--	--	--	--	--
Total Carbohydrate	0.066 (0.092)#	0.284	1	--	--	--	--	--	--
Reducing Sugar	-0.291 (-0.287)#	-0.015	-0.181	1	--	--	--	--	--
Non-reducing Sugar	-0.104 (-0.141)#	0.354	0.24	0.436*	1	--	--	--	--
Total Soluble Sugar	-0.164 (-0.194)#	0.302	0.162	0.628**	0.974**	1	--	--	--
Crude Fibre	-0.04 (-0.047)#	0.002	-0.285	-0.02	-0.339	-0.299	1	--	--
Total Phenol	-0.120 (-0.112)#	-0.016	-0.159	0.675**	0.293	0.423*	-0.311	1	--
Lipid	-0.133 (-0.147)#	0.339	0.201	0.065	0.062	0.07	-0.225	-0.125	1

** Correlation is significant at 1% and * Correlation is significant at 5%.
# Figures in parenthesis are correlation matrix analyzed for peak fruit damage and outside are correlation matrix analyzed for average fruit damage.

sapota, Kirthibarthi (7.74%), CO-2 (9.97%) and Singapore (11.24%) were found to be less infested by seed borer compared to DHS-1 (36.16%) and DHS-2 (32.60%), which differs with present with current results, but damage range is matching.

Seed borer damage under high-density plantations was examined by Khambhu and Bisane (2015) revealed that PKM-3, PKM-4, and CO-3 showed lower 1-2% fruit infestation, while Kalipatti and Cricket Ball recorded the highest fruit damage between 7 and 13% in November and December. Another study by Bisane and Naik (2016) found that, under normal spacing, seed borer fruit loss was lower in PKM-5, DHS-1, PKM-2, Bhuripatti, and PKM-1, ranging from 3 to 5%. In contrast, increased fruit infection, ranging from 8 to 17%, was seen in Kalipatti, Cricket Ball, and CO-2 during November-December. In order to assess sapota for seed borer, Patel (2020) examined the differences between varieties/hybrids in normal and high-density plantations. He showed that Kalipatti, DHS-2 and Cricket Ball had more damage, ranging from 5 to 7%, than PKM-4, PKM-3 and CO-3, which had infestations of less than 3%. In December, Kalipatti and Cricket Ball suffered the most fruit damage.

Looking to other locations for varietal differences, PKM-1 (4.58%) and DSH-1 (9.79%) are the least susceptible, and Kalipatti (18.76%) exhibited high susceptibility against seed borer in Karnataka (Patil and Kumari, 2023). In Tamil Nadu, PKM-1, PKM-2, PKM-1 and Kalipatti were less susceptible and in DHS-1, DHS-2, Kirthibarthi and PKM-4 were more vulnerable to seed borer (AICRP Report, 2019).

In morphological parameters, Kanade (2005) reported that the diameter of infested fruits of different sapota varieties did not play a significant role in pest infestation of the seed borer. While the bigger size of the seed in varieties like DHS-1 and DHS-2 provides sufficient food to the larva, which might be instinctively attracted to the pest. This outcome has some similarity with the present data. In another study, Karthik (2019) noted a significant positive correlation between fruit surface thickness and seed borer damage, but between TSS and total sugar, there was a non-significant positive correlation, which matches the present outcome.

Conclusion

According to the overall varietal evaluation data on sapota, Kirthibarthi, DHS-2, CO-2 and Cricket ball were equally vulnerable to seed borer with the commercial variety Kalipatti. December and April were the critical months for seed borer infestation. However, the tolerance response to seed borer exhibited by Chala collection-1, Chala collection-2, Chala collection-3, Zumakhiya, CO-1, and CO-3 may be due to the escaped nature brought on by the different peak harvesting phases in each variety. Furthermore, no conclusive connection was found between seed borer damage and seed morphology, as well as eight biochemical components. Additionally, one of the useful strategies in upcoming breeding programs will be the adoption of resistant or tolerant cultivars as parent hosts. According to the study, future research should focus on investigating the advanced biochemical properties or attributes of sapota types to better understand their resistance mechanisms against seed borer to maintain the economic impact of incidence on sapota quality.

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RESEARCH ARTICLE

Genetic Variability, Character Association and Path Coefficient Analysis in Satputia (*Luffa acutangula* var. *hermaphrodita*)

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Abstract

A study was carried out to assess the genetic architecture of 36 genotypes of satputia. Fruit yield and fourteen yield contributing traits were recorded from five plants at random. The mean performances of 36 satputia genotypes for yield and its attributing traits showed wide variation. The difference between the genotypic and phenotypic correlation coefficients was narrow for various traits. This indicates lesser influence of the environment in the expression of these traits and the presence of a strong inherent association among the traits. High heritability (%) estimates coupled with high genetic gain (%) were observed for fruit yield per plant (88.27, 72.08), number of fruits per plant (87.83, 53.60), average fruit weight (87.17, 45.41), number of primary branches (91.25, 46.73) and fruit diameter (79.15, 43.13), indicated that these traits are under additive gene effects, hence are more reliable for effective selection. The highest positive significant direct effect on yield was recorded by fruit length, followed by number of clusters per plant, node number of the first hermaphrodite flower and number of fruits/plant at both genotypic and phenotypic levels. Therefore, direct selection for these traits would be worthwhile for the improvement of yield in satputia.

Keywords: Correlation, GCV, *Luffa*, PCV, Path coefficient, Satputia

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Introduction

The rising global demand for nutritious crops has led to an increasing need for exploring underutilised crops and constant breeding programs aimed at producing new and improved varieties that satisfy both societal and economic requirements. Satputia (*Luffa acutangula* var. *hermaphrodita*) is an underutilized vegetable crop belonging to the Cucurbitaceae family. It is grown in the north-eastern part of Uttar Pradesh and adjoining areas as a mixed crop and as a mono-crop in both summer and rainy seasons (Choudhary *et al.*, 2007). It bears hermaphrodite flowers in clusters and is economically important for its tender fruits. Fruits of satputia are nutritious and are good sources of vitamin A, iron, phosphorus, ascorbic acid and zinc.

Despite its economic importance and presence of considerable variability, limited systematic information is available on the genetic characterization of satputia and its genetic improvement. Key genetic parameters, such as genotypic (GCV) and phenotypic (PCV) coefficients of variation,

play a pivotal role in facilitating crop improvement (Das *et al*., 2012). The quantitative characters are very much prone to environmental influence, which necessitates the partitioning of overall variances as heritable and non-heritable components for an efficient breeding programme. To clearly comprehend the link between yield and its components, as well as their direct and indirect consequences, correlation and path analysis are useful tools for understanding the association. Heritability of characters can be relied upon, as it enables the plant breeder to decide the extent of selection pressure to be applied under a particular environment, which separates out the environmental influence from the total variability. The estimation of heritability has a greater role to play in determining the effectiveness of selection for a character, provided it is considered in conjunction with the predicted genetic advance as suggested by Johnson *et al.,* (1955), as the heritability is influenced by biometrical method, generation of hybrid, sample size of experimental material and environment. The findings of this investigation are anticipated to provide important insights into the genetic architecture of satputia for the selection of superior genotypes. This will be accomplished by bridging the information gap between yield and its constituent traits.

Materials And Methods

The investigation was carried out at the research farm of the Division of Vegetable Science of ICAR-Indian Agricultural Research Institute, New Delhi, which lies at 28.63° N, 77.15° E, 228 MSL. The climate of Delhi is semi-arid with hot summers and cool winters. The study material comprised of thirty six germplasm lines of Satputia collected from various parts of India and maintained in the Division of Vegetable Science (Table 1). The experiment was laid out in Randomized Block Design with three replications. Two to three seeds of each genotype were sown in the hills on the ridges at a spacing of 3.5 m × 75 cm. All the recommended practices were followed to grow a successful crop. Fruit yield and fourteen yield contributing traits were recorded (Table 2) from five plants at random.

The statistical analysis for each observed trait under the study was performed using MS-Excel, OPSTAT and Statistical Analysis System (SAS). The mean values of data recorded for the traits for the genotypes were subjected to analysis of variance as described by Gomez and Gomez (1983) for Randomised Block Design. The genotypic and phenotypic coefficients of variability were calculated as per the formulae given by Burton and De-Vane (1953). Heritability in the broad sense was calculated by the formula as suggested by Allard (1960). Genetic gain expressed as per cent ratio of genetic advance and population mean was estimated by the method given by Johnson *et al.,* (1955). The genotypic and phenotypic correlations were calculated as per Al-Jibouri *et al.,* (1958) by using analysis of variance and

Table 1: List of thirty-six genotypes of satputia used in the investigation

Genotype	Source	Genotype	Source
DSat-1	Muzaffarpur, Bihar	DSat-23	Gorakhpur, Uttar Pradesh
DSat-1-2	Muzaffarpur, Bihar	DSat-63	Jhargra, West Bengal
DSat-2	Bhubaneswar, Odisha	DSat-103	Sundergarh, Odisha
DSat-3	Jamshedpur, Jharkhand	DSat-112	Dhenkanal, Odisha
DSat-4	Ranchi, Jharkhand	DSat-112-1	Angul, Odisha
DSat-4-1	Madhubani, Bihar	DSat-113	Midnapore, West Bengal
DSat-4-12	Begusarai, Bihar	DSat-115	Midnapore, West Bengal
DSat-5	Koderma, Jharkhand	DSat-116	Jamshedpur, Jharkhand
DSat-7	Hazaribagh, Jharkhand	DSat-120	Sambalpur, Odisha
DSat-8	Pusa, Bihar	DSat-121	Hajipur, Bihar
DSat-9	Gumla, Jharkhand	DSat-122	Barabanki, Uttar Pradesh
DSat-9-1	Sitamarhi, Bihar	DSat-123	Ranchi, Jharkhand
DSat-10	Varanasi, Uttar Pradesh	DSat-124	Tezu, Arunachal Pradesh
DSat-11	Samastipur, Bihar	DSat-125	Purulia, West Bengal
DSat-11-1	Samastipur, Bihar	DSat-126	Dewariya, Uttar Pradesh
DSat-14	Gaya, Bihar	DSat-128	Bahraich, Uttar Pradesh
DSat-15	Barauni, Bihar	Kashi Khushi	IIVR, Varanasi
DSat-19	Sambalpur, Odisha	Swarna Sawani	ICAR Complex for Eastern Region Regional Center, Ranchi

Table 2: ANOVA and estimates of genetic variability for yield and its attributing traits in satputia genotypes

Trait	Range	Mean	Coefficient of variability (%)		Broad sense heritability	Genetic advance	Mean sum of square		
	Min-Max		GCV (%)	PCV (%)	(%) of mean	as (%)	Replication	Genotype	Error
Days to first female flower anthesis	33.75-50.17	40.64	12.58	12.76	97.21	32.75	0.27	2761.57**	52.29
Days to 50% flowering	36.74-55.00	43.98	8.50	9.14	86.36	20.84	0.73	3315.81**	153.66
Days to first fruit set	38.80-56.19	48.06	10.71	11.20	91.32	27.01	4.19	2872.12**	176.4
Node number of first hermaphrodite flower	4.08-8.09	5.77	14.85	22.52	43.00	25.84	6.72	109.82**	66.44
Number of primary branches	3.13-6.58	4.49	18.53	19.40	91.25	46.73	0.07	74.37**	4.6
Vine length (m)	1.30-2.77	1.89	16.45	25.35	42.07	28.16	0.55	14.70**	9.25
Number of fruits per cluster	3.10-6.95	5.38	8.44	19.93	17.95	9.45	2.01	55.03**	66.44
Number of clusters per plant	2.36-4.98	3.93	13.93	20.98	44.04	24.40	22.01	45.11**	26.84
Days to first fruit harvest	44.66-60.33	54.10	6.87	7.56	82.66	16.50	23.35	1559.03**	203.74
Fruit length (cm)	6.09-9.83	8.41	9.92	15.70	39.96	16.56	3.63	110.44**	73.7
Fruit diameter (mm)	15.93-32.37	22.28	18.37	20.64	79.15	43.13	5.25	1895.24**	305.97
Average fruit weight (g)	43.17-96.53	60.88	18.42	19.73	87.17	45.41	38.84	13628.41**	1274.66
Number of fruits/ plant	9.80-25.57	17.31	21.66	23.12	87.83	53.60	75.94	1541.90**	136.1
100 Seed weight (g)	6.53-13.53	8.85	15.76	18.84	69.98	34.80	0.40	229.98**	57.54
Fruit yield per plant (kg)	0.57-2.06	1.05	29.06	30.93	88.27	72.08	164.11	10007.67**	849.24

**Significant at 1% level of probability

covariance matrix in which total variability was split into replications, genotypes and errors. The genotypic and phenotypic correlation coefficients were used to find out their direct and indirect contribution towards yield per plot. The direct and indirect paths were obtained by following Dewey and Lu (1959).

Results and Discussion

The improvement in crop yield depends upon the extent of genetic variability available in breeding materials and the extent to which the major yield contributing traits are heritable from generation to generation. The genetic variability can be a choice for selecting suitable parents. Analysis of variance showed that the mean sum of squares for genotypes was highly significant for all traits, indicating the existence of a high degree of genetic variability in the germplasm. Nevertheless, the analysis of variance is not sufficient and conclusive to elucidate all the inherent genotypic variance in the collections. Therefore, it becomes necessary to split the observed variability into phenotypic variation (PV) and genotypic variation (GV) and subsequently GCV and PCV, which indicates the extent of variability existing for various traits. Moderate GCV and PCV variation (>20%) were observed for fruit yield per plant (kg) and number of fruits per plant (Table 2). Similar results were also obtained by Karuppaiah *et al.,* (2002) in ridge gourd. It indicated the presence of moderate variability in the germplasm for selection, and further, the differences between PCV and GCV values were minimum indicating that the traits under study were less influenced by the environment. Hence, these characters can be relied upon, and simple selection can be practiced for further improvement.

Table 3: Phenotypic and genotypic correlations among different quantitative traits in thirty-six genotypes of satputia

Traits		DFA	DFS	NNHF	NPB (m)	VL	NOFC	NCPP	DTFFH (cm)	FL (mm)	FD (g)	AFW	NOFPP (g)	100 SW (kg)	FYPP
D50F	(P)	0.989*	0.653*	0.215*	-0.004	0.033	0.18*	-0.05	0.536*	-0.111	-0.040	-0.035	-0.163*	-0.164*	-0.266*
	(G)	0.960*	0.629*	0.143*	0.011	0.060	0.11*	-0.06	0.506*	-0.059	-0.030	-0.007	-0.146*	-0.134*	-0.199*
DFA	(P)		0.692*	0.194*	0.032	0.067**	0.22*	-0.04	0.545*	-0.081	-0.038	-0.005	-0.158*	-0.139*	-0.264*
	(G)		0.671*	0.136*	0.051	0.099**	0.13*	-0.03	0.529*	-0.055	-0.031	0.008	-0.148*	-0.124*	-0.205*
DFS	(P)			0.047	0.174*	-0.008	0.130	-0.05	0.890**	0.073	-0.120*	0.087	-0.064	-0.009	-0.173*
	(G)			0.039	0.171*	-0.026	0.07	-0.04	0.825*	0.043	-0.111*	0.077	-0.060	-0.010	-0.152*
NNHF	(P)				-0.412**	0.072*	-0.17**	-0.44**	0.138*	0.096*	0.048	0.269*	-0.390**	-0.176*	0.101*
	(G)				-0.281*	0.118*	-0.10**	-0.22*	0.115*	0.195*	0.037	0.245*	-0.313*	-0.123*	0.137*
NPB	(P)					-0.105*	0.44*	0.04	0.064	0.063	-0.034	0.075	0.090**	0.189*	-0.325*
	(G)					-0.153*	0.36*	0.06	0.050	0.021	-0.048	0.057	0.092**	0.175*	-0.325*
VL(m)	(P)						-0.17**	-0.10	-0.040	-0.379*	0.090**	-0.006	0.042	0.033	-0.116*
	(G)						-0.10**	0.03	-0.061	-0.216*	0.095**	-0.020	0.060	0.043	-0.151*
NOFC	(P)							0.18**	0.010	0.041	-0.254*	-0.204*	0.451**	0.293*	-0.217**
	(G)							0.10**	0.008	0.073	-0.137*	-0.112*	0.346*	0.238*	-0.087**
NCPP	(P)								-0.151*	0.125	-0.011	-0.018	0.635**	0.490**	0.135*
	(G)								-0.170*	0.029	-0.052	0.012	0.511*	0.408*	0.132*
DTFFH	(P)									0.115	-0.075	0.098	-0.130*	-0.055	-0.086*
	(G)									0.076	-0.064	0.077	-0.122*	-0.060	-0.116*
FL(cm)	(P)										-0.056	0.237*	0.086	0.175**	0.257*
	(G)										-0.045	0.165*	0.044	0.101**	0.149*
FD(mm)	(P)											0.270*	-0.413**	-0.166*	-0.073*
	(G)											0.229*	-0.370*	-0.155*	-0.104*
AFW (g)	(P)												-0.095**	0.561*	-0.002
	(G)												-0.090**	0.561*	-0.022
NOFPP	(P)													0.751*	0.097*
	(G)													0.754*	0.104*
100SW(g)	(P)														0.055
	(G)														0.056

*Significant at 5% level of probability; **Significant at 1% level of probability (P: Phenotypic correlation; G: Genotypic correlation)

D50F: Days to 50% flowering, DFA: Days to first female flower anthesis, DFS: Days to first fruit set, NNHF: Node number of first hermaphrodite flower, NPB: Number of primary branches, VL: Vine length (m), NOFC: Number of fruits per cluster, NCPP: Number of clusters per plant, DTFFH: Days to first fruit harvest, FL: Fruit length (cm), FD: Fruit diameter (mm), AFW: Average fruit weight (g), NOFPP: Number of fruits/ plant, 100 SW: 100 seed weight (g), FYPP: Fruit yield per plant (kg).

Table 4: Path coefficient analysis for different traits in satputia genotypes

Trait	D50F	DFA	DFS	NNHF	NPB	VL (m)	NOFC	NCPP	DTFFH	FL (cm)	FD (mm)	AFW (g)	NOFPP	100 SW (g)	Phenotypic correlation with fruit yield
Days to 50% flowering	-1.710	1.580	-0.177	0.042	0.001	-0.006	-0.060	-0.006	0.125	-0.019	0.001	0.023	0.064	-0.124	-0.266*
Days to first female flower anthesis	-1.691	1.597	-0.188	0.038	-0.007	-0.013	-0.072	-0.005	0.127	-0.013	0.001	0.003	0.062	-0.105	-0.264*
Days to first fruit set	-1.116	1.105	-0.272	0.009	-0.038	0.002	-0.043	-0.006	0.208	0.012	0.004	-0.056	0.025	-0.007	-0.173*
Node number of first hermaphrodite flower	-0.368	0.310	-0.013	0.196	0.090	-0.013	0.057	-0.051	0.032	0.016	-0.001	-0.175	0.153	-0.133	0.101*
Number of primary branches	0.008	0.051	-0.047	-0.081	-0.218	0.020	-0.147	0.004	0.015	0.011	0.001	-0.048	-0.035	0.143	-0.325*
Vine length (m)	-0.057	0.107	0.002	0.014	0.023	-0.187	0.056	-0.011	-0.009	-0.063	-0.003	0.004	-0.016	0.025	-0.116*
Number of fruits per cluster	-0.311	0.344	-0.035	-0.034	-0.096	0.031	-0.333	0.021	0.002	0.007	0.008	0.133	-0.177	0.222	-0.217**
Number of clusters per plant	0.089	-0.065	0.013	-0.086	-0.008	0.018	-0.061	0.117	-0.035	0.021	0.000	0.012	-0.250	0.370	0.135*
Days to first fruit harvest	-0.916	0.870	-0.242	0.027	-0.014	0.007	-0.003	-0.018	0.234	0.019	0.002	-0.064	0.051	-0.042	-0.086*
Fruit length (cm)	0.190	-0.129	-0.020	0.019	-0.014	0.071	-0.014	0.015	0.027	0.167	0.002	-0.154	-0.034	0.132	0.257*
Fruit diameter (mm)	0.068	-0.061	0.033	0.010	0.007	-0.017	0.085	-0.001	-0.018	-0.009	-0.031	-0.176	0.163	-0.125	-0.073*
Average fruit weight (g)	0.060	-0.008	-0.024	0.053	-0.016	0.001	0.068	-0.002	0.023	0.040	-0.008	-0.650	0.038	0.424	-0.002
Number of fruits/plant	0.279	-0.252	0.017	-0.077	-0.020	-0.008	-0.150	0.074	-0.031	0.014	0.013	0.062	-0.393	0.568	0.097*
100 Seed weight (g)	0.280	-0.222	0.003	-0.035	-0.041	-0.006	-0.098	0.057	-0.013	0.029	0.005	-0.364	-0.296	0.756	0.055

Residual effect: 0.69, Direct effect= Bold diagonals.

*Significant at 5% level of probability; **Significant at 1% level of probability

D50F: Days to 50% flowering, DFA: Days to first female flower anthesis, DFS: Days to first fruit set, NNHF: Node number of first hermaphrodite flower, NPB: Number of primary branches, VL: Vine length (m), NOFC: Number of fruits per cluster, NCPP: Number of clusters per plant, DTFFH: Days to first fruit harvest, FL: Fruit length (cm), FD: Fruit diameter (mm), AFW: Average fruit weight (g), NOFPP: Number of fruits/ plant, 100 SW: 100 seed weight (g).

Lower difference of PCV over GCV were observed in satputia genotypes for traits, *viz*., days to first female flower anthesis, days to 50% flowering, days to first fruit set, days to first fruit harvest, number of primary branches, fruit length, fruit diameter, average fruit weight, number of fruits per plant, 100 seed weight and fruit yield per plant, thereby indicating that these traits were less influenced by environment and mostly attributed to genotype. Thus, phenotypic variability may be a good measure of genotypic variability for these traits in satputia. Similar results were also obtained by Koppad *et al.,* (2015) in ridge gourd.

High heritability (>60%) estimates along with high genetic advance as a percentage of mean (>40%) were recorded for fruit yield per plant, number of fruits per plant, average fruit weight, number of primary branches and fruit diameter, thereby indicating the predominance of additive gene component. Thus, there is ample scope for improving these traits with direct selection. Besides, high heritability coupled with moderate genetic gain was noted for days to first female flower anthesis, days to 50% flowering, days to first fruit set and days to first fruit harvest, indicating that these traits are under non-additive gene effects and selection for these traits will be less effective. Similar findings were also reported by Sureja *et al.,* (2010) in ash gourd, Kumar *et al.,* (2013) in sponge gourd and Koppad *et al.,* (2015) in ridge gourd.

Variability studies provide information on the extent of improvement to be achieved in different traits, but they do not throw light on the extent and nature of the relationship existing between various characters. Therefore, for a rational approach towards the improvement of yield, selection has to be made for the components of yield, since there may not be genes for yield *per se*, but only for various yield components (Grafius, 1959). Further, many of these yield contributing characters may interact in desirable and undesirable directions. Hence, knowledge regarding the association of various characters among themselves and with economic characters is essential. In the present study, the genotypic and phenotypic correlation coefficients were worked out for yield and its attributing components. The difference between the genotypic and phenotypic correlation coefficients was narrow for various traits in the present findings, and this indicates the lesser influence of environment in the expression of these traits and the presence of strong inherent association among the traits (Table 3).

Fruit yield per plant was found to be positively and significantly (at P=0.05) associated with node number of first hermaphrodite flower (rg= 0.137), number of clusters per plant (rg= 0.132), fruit length (rg= 0.149) and number of fruits per plant (rg= 0.104) at the genotypic level. Hence, direct selection for these traits could be made for improving yield. Based on the overall results of phenotypic and genotypic correlation, it is possible to breed desirable satputia varieties combining high fruit yield with superior quality attributes.

The analysis of correlation indicates the association pattern of component traits with yield, and it simply represents the general association of a particular trait with yield rather than providing cause and effect relationship. The technique of path coefficient analysis, developed by Wright (1921) and demonstrated by Dewey and Lu (1959), facilitates partitioning the correlation coefficients into direct and indirect involvement of various characters on yield. As such, it measures the direct influence of one variable upon another. Such information would be of immense value in enabling the breeder to exclusively identify important component traits of yield and utilize the genetic stock for improvement in a planned way. In the present investigation, 15 traits were selected for path analysis, and the traits that showed significant positive correlation with yield are fruit length (0.257), number of clusters per plant (0.135), node number of first hermaphrodite flower (0.101), and number of fruits per plant (0.097) (Table 4). Therefore, direct selection for these traits would be worthwhile for the improvement of yield in satputia.

Conclusion

In this study, the variability parameters, heritability, genetic advance, correlation and path analysis for yield and yield contributing traits were assessed in thirty-six genotypes of satputia. The results indicated a moderate to high range of variability in the majority of the traits. High heritability estimates along with high genetic advance as a percentage of the mean were recorded for fruit yield per plant, number of fruits per plant, average fruit weight, number of primary branches and fruit diameter, thereby indicating the predominance of additive gene component. Fruit yield per plant was found to be positively and significantly associated with a number of yield contributing traits, thereby indicating that direct selection of these traits will be effective for yield improvement in satputia.

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RESEARCH ARTICLE

Exploring Genetic Diversity and Yield-Contributing Traits in Sorghum (*Sorghum bicolor*) using D^2 Statistics and Principal Component Analysis

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Abstract

Sorghum is a vital cereal crop with immense potential for grain and fodder, especially in semi-arid regions. This study evaluated the genetic diversity among 96 sorghum genotypes using Mahalanobis D^2 statistics and principal component analysis to identify key traits contributing to yield and other agronomic characteristics. The field experiment was conducted at the Millet breeding station, TNAU, Coimbatore, with two replications. The genotypes were grouped into 10 clusters, with distinct clusters showing unique trait combinations valuable for targeted breeding goals. For example, genotypes such as IS 28517, IS 29114, and TNSS 223 are ideal for biomass production because of their tall, late-maturing traits, whereas IS 10939 and AS 3883 are promising for high seed weight and high yield, respectively. Principal component analysis (PCA) identified traits such as leaf width, panicle weight, plant height, and flag leaf length as major contributors to genetic variability. The distribution of genotypes in the PCA biplot highlighted specific associations between yield-related traits and genotype groupings, offering insights for selecting parents for hybridization. This research provides valuable information for sorghum breeding programs aimed at enhancing productivity and resilience in dryland regions, thereby supporting food security and sustainable agriculture.

Keywords: Genetic diversity, Genetic resources, Hybridization, Multivariate analysis, Sorghum

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench], the fifth most vital cereal crop worldwide, originated and was domesticated in northeastern Africa, with some of the earliest evidence of its domestication found in eastern Sudan around the 4th millennium BC. From there, it spread to other regions like India, China and eventually to North America. This widely cultivated species has been classified into five primary races—bicolor, durra, kafir, guinea, and caudatum—and ten intermediate races, reflecting its broad genetic diversity and adaptability (Harlan and de Wet, 1972). In India, sorghum is grown during both the *kharif* and *rabi* seasons, serving a dual purpose by providing both grain and fodder to farming communities (Swamy *et al*., 2018). Rabi-season sorghum is particularly valued for its superior grain quality, as it is primarily grown for human consumption and has a higher market price than kharif sorghum (Prasad *et al*., 2020). Sorghum is the fifth most significant cereal crop in India, after wheat, rice, maize and pearl millet and sorghum is cultivated extensively in semiarid, rainfed

regions. The major sorghum-producing states include Maharashtra, Karnataka, Tamil Nadu, Rajasthan, and Madhya Pradesh. Sorghum displays substantial variability in plant morphology, panicle and grain characteristics, and physiological responses to selection pressures and is strongly influenced by environmental factors (Ezeaku *et al*., 1997). Given that rice and wheat are staple foods for much of the global population, sorghum, as a C4 crop, holds great potential for dryland agriculture, optimizing dryland usage and increasing farmer incomes (Dhar *et al*., 2024). Known as "the king of coarse cereals" and a "nutri-cereal," sorghum has a remarkable nutritional profile and is rich in unsaturated fats, proteins, fibre, and minerals such as potassium, phosphorus, calcium, and iron (Thant *et al*., 2020).

Understanding the genetic diversity of a crop is crucial for breeders, as it allows for the selection of appropriate parental lines for breeding programs and the introduction of genes from more distantly related germplasms (Bucheyeki *et al*., 2009). In general, parents that exhibit greater genetic divergence are expected to produce hybrids with greater heterotic potential and create a broad spectrum of variability in segregating generations (Prasad and Sridhar, 2019). To analyse genetic variation, D^2 statistics are used to classify genotypes and measure genetic distance among clusters. Principal component analysis (PCA) further aids in identifying the most yield-contributing agronomic traits, which can then be emphasized in breeding efforts. Consequently, the present study aimed to evaluate genetic diversity among sorghum genotypes and identify the key traits that contribute to grain yield.

Materials and Methods

The experimental material consisted of 96 diverse sorghum genotypes, including two checks (CO 32 and K 13) collected from the Department of Millets, TNAU, Coimbatore and ICRISAT Hyderabad (Table 1). These genotypes were selected to represent wide genetic variability in terms of geographical origin, agro-morphological traits, maturity duration, and yield potential. The inclusion of landraces, breeding lines, exotic accessions, and two standard checks ensured adequate diversity for assessing genetic divergence and identifying superior parents for breeding programmes. The field experiment was carried out in a randomized block design (RBD) with two replications at Millets Breeding Station, TNAU, Coimbatore, between 11°N latitude and 77°E longitude during Rabi 2023-24. Each line was sown in 4-meter rows with 45 cm x 15 cm spacing. All the recommended agronomical practices for sorghum were followed to raise a good crop during the season. Five randomly selected plants of each genotype were observed for fourteen yield and yield-contributing traits, i.e., days to 50% flowering (DFF), number of leaves per plant (NL), leaf length (LL), leaf width (LW), flag leaf length (FLL), flag leaf width (FLW), plant height (PH), stem girth (SG), panicle length (PL), panicle width (PW), panicle weight (PWT), days to maturity (DM), hundred seed weight (HSW), and single-plant yield (SPY). The recorded data were then subjected to statistical analysis. To assess the genetic diversity present in the sorghum accessions, Mahalanobis' D^2 statistics were performed in WINDOSTAT ver. 7.1, and multivariate principal component analysis was performed in the AGRICOLAE package of R software.

Table 1: List of the ninety-six sorghum genotypes evaluated in the study

Entries	Origin	Entries	Origin
T Kallupatti local	India	SD 2033	-
IS 22794	Ethiopia	IS 23460	Yemen
IS 29640	USA	IS 30383	China
IS 10718	Ethiopia	IS 30450	India
IS 29690	India	IS 30507	Korea
IS 20594	Ethiopia	IS 1212	China
CO 4	India	IS 1233	China
IS 1052	India	IS 10477	USA
Erichanampatti local	India	IS 3937	India
IS 1141	Sudan	IS 3971	India
ICSB 23	India	IS 5286	India
SH 92	India	IS 5603	India
SH 29	India	IS 10304	India
SH 25	India	IS 10472	Ethiopia
N 14	Sudan (also referred as IS 18331)	IS 10939	USA

SH 24	Kenya	IS 19003	Ethiopia
IS 1137	India	IS 19534	Sudan
SH 16	India	IS 19824	India
E 96	Ethiopia	IS 21770	Sudan
SEB 12002	India	CO 32	India
A 6072	Ethiopia	APK 1	India
AS 3883	India	K 13	India
IS 9283	India	M35-1	India
CO 26	India	IS 1219	China
IS 3076	Deccan region of India	SD 2033	Sudan
Kottathur local	India	IS 23460	India
IS 30536	Korea	IS 30383	China
SD 12885	USA	IS 30450	China
CS 113	Sudan	IS 30507	Republic of Korea
SD 8375	Sudan	IS 1212	China
Paiyur 2	India	IS 1233	China
IS 2883	Italy	IS 10477	USA
IS 12804	Turkey	IS 3937	USA
IS 24578	Lebanon	IS 3971	India
IS 24617	Lebanon	IS 5286	India
IS 24629	Lebanon	IS 5603	India
IS 24632	Lebanon	IS 10304	USA
IS 28517	Yemen	IS 10472	USA
IS 29114	Yemen	IS 10939	USA
IS 511	Mexico	IS 19003	Sudan
IS 869	USA	IS 19534	Sudan
IS 932	Sudan	IS 19824	India
IS 2940	USA	IS 21770	Sudan
IS 3932	USA	CO 32	India
RS 29	India	APK 1	India
RC 585	-	K 13	India
IS 20575	Sudan	M35-1	India
IS 27786	Morocco	IS 1219	China

Results and Discussion

D^2 analysis

According to the Mahalanobis D^2 analysis, the D^2 values measure the degree of diversity and determine the pertinent contribution of each constituent character to total divergence. The genotypes studied were grouped into different clusters on the basis of genetic distance among the genotypes.

In this study, 96 genotypes, including two checks, were grouped into 10 different clusters using Tocher's method (Fig. 1) on the basis of their relative distances, i.e., D^2 values. The dendrogram can be a useful visualization tool for understanding the structure of the data and identifying natural groupings among the observations. Among the 10 clusters, a large number of genotypes (64) fell into Cluster I, implying greater similarity among these genotypes, which indicates a lack of sufficient divergence within this group. Cluster II consisted of 16 genotypes, followed by cluster V with 7 genotypes and cluster III with 3 genotypes. In contrast, the presence of solitary clusters (III, IV, VI, VII, VIII, and X) containing only one genotype in each cluster suggested that these genotypes are quite distinct from the others, potentially harbouring unique traits that could be valuable for breeding programs (Table 2).

Table 2: Clustering of ninety-six sorghum genotypes by Tocher's method into 10 different clusters

Clusters	Number of genotypes	Genotypes
Cluster I	64	IS 2883, IS 23460, IS 22794, SEB 12002, IS 20575, IS 19003, IS 19824, ICSB 23, Erichanampatti local, IS 3971, IS 1141, Kottathur local, CO 4, T Kallupatti local, IS 20594, E 96, SD 12885, IS 1052, CO 32, IL 46, A 6072, IS 27786, MR 99, IS 10477, IS 511, IS 10472, M35-1, IS 3932, ICS PIRMER, IS 2940, K

		13, ART 3015, IS 29690, SH 16, IS 24629, IL 497, IS 19534, IS 869, COS 28, IS 932, IS 10304, SH 92, SH 25, Paiyur 2, CO 30, IS 3937, IS 5603, SD 8375, IS 1219, IS 30450, IS 30507, IS 5286, IS 21770, SH 24, IS 1212, ISHAT 2001, SD 2033, IS 24617, IS 24632, IS 24578, AKZ 354, IS 1233, IS 3076, N 14
Cluster II	16	TNS 695, TNS 704, CO 26, TNS 702, IS 29322, APK 1, TNSS 225, TNSS 227, IS 1137, IS 9283, SAR 39, SH 29, CS 113, A 404, RS 29, SB 103
Cluster III	1	IS 30536
Cluster IV	1	IS 30383
Cluster V	7	IS 29640, ICSB 29, IS 12804, CO 27, TNFS 230, TNFS 239, IS 10718
Cluster VI	1	IS 10939
Cluster VII	1	RS 627
Cluster VIII	1	RC 585
Cluster IX	3	IS 28517, IS 29114, TNSS, 223
Cluster X	1	AS 3883

The mean values of ten clusters for 96 genotypes (Table 3) revealed notable genetic diversity across traits, which is valuable for breeding objectives. Cluster IX presented the highest means for plant height (252.85 cm), days to 50% flowering (75.83 days), number of leaves per plant (10.67), flag leaf length (64.43 cm), and days to maturity (113.83 days). Thus, the genotypes present in this cluster, such as IS 28517, IS 29114, and TNSS 223, were late-maturing, tall, and had more leaves, making them promising for improving the biomass of forage and silage crops. Cluster VI had the highest hundred-seed weight (4.25 g), indicating that genotype IS 10939 has strong grain yield potential. Cluster X presented the greatest means for leaf length (87.12 cm), stem girth (2.15 cm), panicle weight (102.30 g), and single-plant yield (78.24 g), identifying genotype AS 3883 as ideal for high-yield, dual-purpose varieties for grain and fodder (Greater plant height- 224.5 cm). Cluster VII presented low values for days to 50% flowering, panicle length, panicle width, and single-plant yield, indicating early maturity and suitability for short-duration crops. Cluster VIII exhibited maximum mean values for leaf width (10.50 cm) and flag leaf width (9.47 cm), which indicated the potential for increased photosynthesis and biomass. Cluster IV had the highest mean panicle length (32.67 cm), and the solitary nature of this cluster suggested that the genotype within this cluster was quite distinct and could possess unique traits not found in the other clusters. Hence, the genotypes linked with these clusters can be utilized as parents in a breeding plan, as observed by Navya *et al*., (2021) in a similar study.

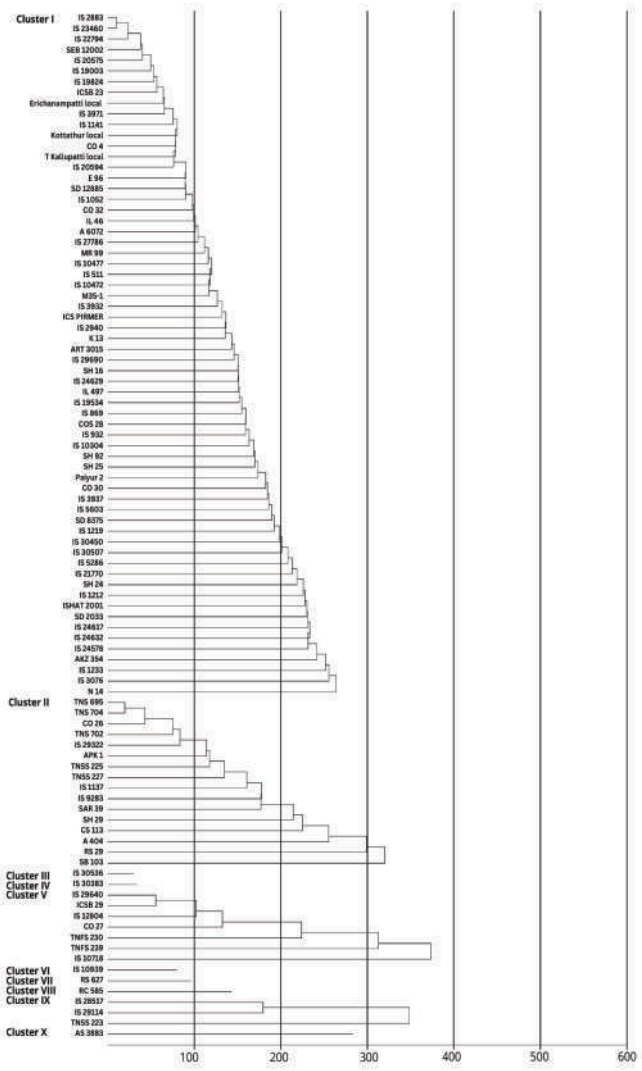

Fig. 1: Dendrogram showing the clustering pattern of 96 genotypes by Tocher's method.

The analysis of intracluster and intercluster distances offers valuable insights for breeding strategies (Table 4). The highest intracluster distance in Cluster IX (20.22) highlighted considerable variability, indicating potential for selection within this group containing the genotypes IS 28517, IS 29114, and TNSS, 223. Clusters with zero intracluster distances, such as III, IV, VI, VII, VIII, and X, represent solitary clusters. The largest intercluster distances were observed between Clusters VI and X (46.91) and between Clusters VII and X (44.71), suggesting significant genetic divergence. Therefore, IS 10939, AS 3883 and RS 627 could be selected as parental materials for hybridization programs. The overall larger intercluster distances than intracluster distances suggested substantial diversity across groups, which aligns with the findings of Prasad and Biradar (2018), Thant *et al.*, (2020), Navya *et al*., (2021), Verma *et al*., (2022) and Bhadouriya and Bhadouriya (2024).

Table 3: Cluster mean values of 10 clusters for 14 different biometrical characters

	DFF	NL	LL	LW	FLL	FLW	PH	SG	PL	PW	PWT	DM	HSW	SPY
CLUSTER I	62.50	7.02	57.30	5.81	37.84	4.88	157.40	1.12	18.97	5.58	30.57	104.98	2.50	20.42
CLUSTER II	61.94	7.86	72.27	7.58	45.57	6.37	173.36	1.28	24.66	6.50	63.58	105.59	2.55	45.96
CLUSTER III	56.50	5.66	52.53	5.35	38.93	4.49	211.79	1.11	31.42	5.35	14.88	99.50	1.87	10.34
CLUSTER IV	66.50	7.16	56.13	5.22	40.90	3.80	191.75	1.18	32.67	5.15	30.33	109.50	2.05	22.93
CLUSTER V	55.64	6.69	56.41	4.89	38.99	4.58	198.11	1.10	28.05	8.15	19.73	97.36	1.64	13.89
CLUSTER VI	59.50	7.34	42.92	4.07	27.45	3.15	103.89	1.09	19.00	4.91	23.20	104.50	4.25	17.99
CLUSTER VII	51.50	8.66	78.92	7.40	48.30	7.37	106.33	1.12	17.78	3.68	17.25	91.50	1.45	9.00
CLUSTER VIII	73.50	8.84	77.05	10.50	59.19	9.47	118.89	1.38	27.05	9.52	92.90	113.50	2.46	57.70
CLUSTER IX	75.83	10.67	79.59	9.23	64.43	8.10	252.85	2.06	20.33	9.34	65.61	113.83	3.36	37.87
CLUSTER X	65.50	7.66	87.12	10.12	53.30	7.74	224.50	2.15	31.13	8.05	102.30	105.50	2.62	78.24

Note: DFF=Days to 50% flowering (days count), NL=number of leaves per plant (numbers count), LL=leaf length (cm), LW=leaf width (cm), FLL=flag leaf length (cm), FLW=flag leaf width (cm), PH=plant height (cm), SG=stem girth (cm), PL=panicle length (cm), PW=panicle width (cm), PWT=panicle weight (g), DM=days to maturity (days count), HSW=hundred seed weight (g/100 seed), and SPY=single plant yield (g).

Table 4: Average intracluster and intercluster distances (D^2 values) among the 10 clusters

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X
Cluster I	13.54	21.09	17.48	16.86	20.88	18.88	17.95	29.49	33.14	40.44
Cluster II	21.09	15.09	24.62	19.87	27.01	27.32	25.67	19.74	28.46	27.34
Cluster III	17.48	24.62	0.00	9.12	15.82	25.65	17.77	35.45	38.73	42.29
Cluster IV	16.86	19.87	9.12	0.00	19.03	24.01	19.80	30.37	35.40	37.16
Cluster V	20.88	27.01	15.82	19.03	17.60	29.55	21.42	34.39	38.36	43.68
Cluster VI	18.88	27.32	25.65	24.01	29.55	0.00	27.73	35.69	36.04	46.91
Cluster VII	17.95	25.67	17.77	19.80	21.42	27.73	0.00	34.54	40.46	44.71
Cluster VIII	29.49	19.74	35.45	30.37	34.39	35.69	34.54	0.00	25.82	24.51
Cluster IX	33.14	28.46	38.73	35.40	38.36	36.04	40.46	25.82	20.22	29.65
Cluster X	40.44	27.34	42.29	37.16	43.68	46.91	44.71	24.51	29.65	0.00

Principal component analysis

A multivariate statistical approach known as principal component analysis (PCA) was performed for 96 sorghum genotypes across 14 biometric traits. PCA was used to assess the genetic diversity of the sorghum genotypes and their relationships with the observed attributes on the basis of the correlation between the traits and the pattern of variation in the genotypes. A scree plot displays the eigenvalues and percentage of variation associated with each principal component (Fig. 2). Three major principal components, i.e., PC1, PC2 and PC3, were extracted because they had eigenvalues greater than one (Brejda *et al*., 2000), which collectively explained 67.38% of the total variation (Table 5). PC1 contributed 44.77% of the variance and was heavily influenced by traits such as leaf width, panicle weight, and plant height, indicating that these traits are key drivers of genetic diversity. PC2, accounting for 12.36%, was strongly associated with panicle length and width, indicating that panicle size plays a significant role in genotype differentiation. PC3 explained 10.25% of the variance and was influenced mainly by plant height, days to flowering, and panicle dimensions.

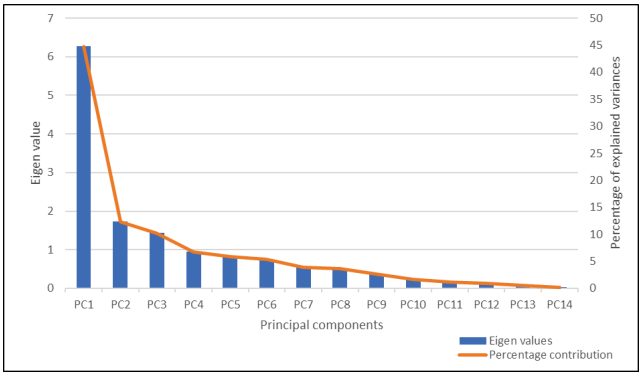

Fig. 2: Scree plot showing the eigenvalue and percentage contribution of each principal component towards divergence.

Similarly, Pugahendhi *et al.*, (2023) reported that the first three principal components accounted for 43.26% of the total variation and highlighted the key agro-morphological traits driving diversity among 96 kharif sorghum genotypes using 20 agro-morphological traits. However, the factor loadings of traits on each principal component (PC) provided insights into how each trait contributes to the observed variability among the genotypes (Table 5). The traits with positive loadings on PC1—such as leaf width, flag leaf width, panicle weight, flag leaf length, single plant yield, stem girth, days to 50% flowering, and plant height—were key drivers of genetic divergence among the sorghum genotypes, highlighting their importance for phenotypic variability. Genotypes with higher values

Table 5: Relative contribution and factor loading of each biometrical trait towards divergence in the 3 major principal components

	Principal components		
Variables	Pc1	Pc2	Pc3
Eigen values	6.268	1.731	1.435
Variance %	44.773	12.364	10.253
Cumulative variance %	44.773	57.137	67.39
Trait	**Factor loadings**		
DFF	0.229	-0.413	0.384
NL	0.221	-0.210	-0.054
LL	0.328	0.165	-0.161
LW	0.359	0.043	-0.159
FLL	0.335	0.095	0.143
FLW	0.346	0.120	-0.089
PH	0.119	0.232	0.568
SG	0.287	-0.05	0.088
PL	0.112	0.343	0.181
PW	0.206	0.339	0.288
PWT	0.341	0.044	-0.295
DM	0.215	-0.47	0.321
HSW	0.123	-0.466	-0.162
SPY	0.318	0.065	-0.327

Note: DFF=Days to 50% flowering, NL=number of leaves per plant, LL=leaf length, LW=leaf width, FLL=flag leaf length, FLW=flag leaf width, PH=plant height, SG=stem girth, PL=panicle length, PW=panicle width, PWT=panicle weight, DM=days to maturity, HSW=hundred seed weight, and SPY=single plant yield.

for these traits showed strong associations with PC1 variance. The results were in accordance with the findings of Pugahendhi *et al*., (2023) for traits such as days to 50% flowering, plant height, and leaf dimensions; by Boukrouh *et al*., (2023) for grain yield, panicle weight, and plant height; and by Malini *et al*., (2023) for flowering and maturity timing. Overall, flag leaf length, plant height, panicle length, and panicle width presented positive loadings across all three principal components, indicating that they are key contributors to genetic divergence and phenotypic differentiation among sorghum genotypes. The consistent influence of these genes suggested that they are valuable traits for selection in sorghum breeding programs.

PCA also provided valuable insights into the relationships among biometric traits (Fig. 3), as shown by vector angles. An acute angle between days to 50% flowering and days to maturity indicated a strong positive correlation, which is useful for selecting early-maturing types. Similarly, positive correlations were

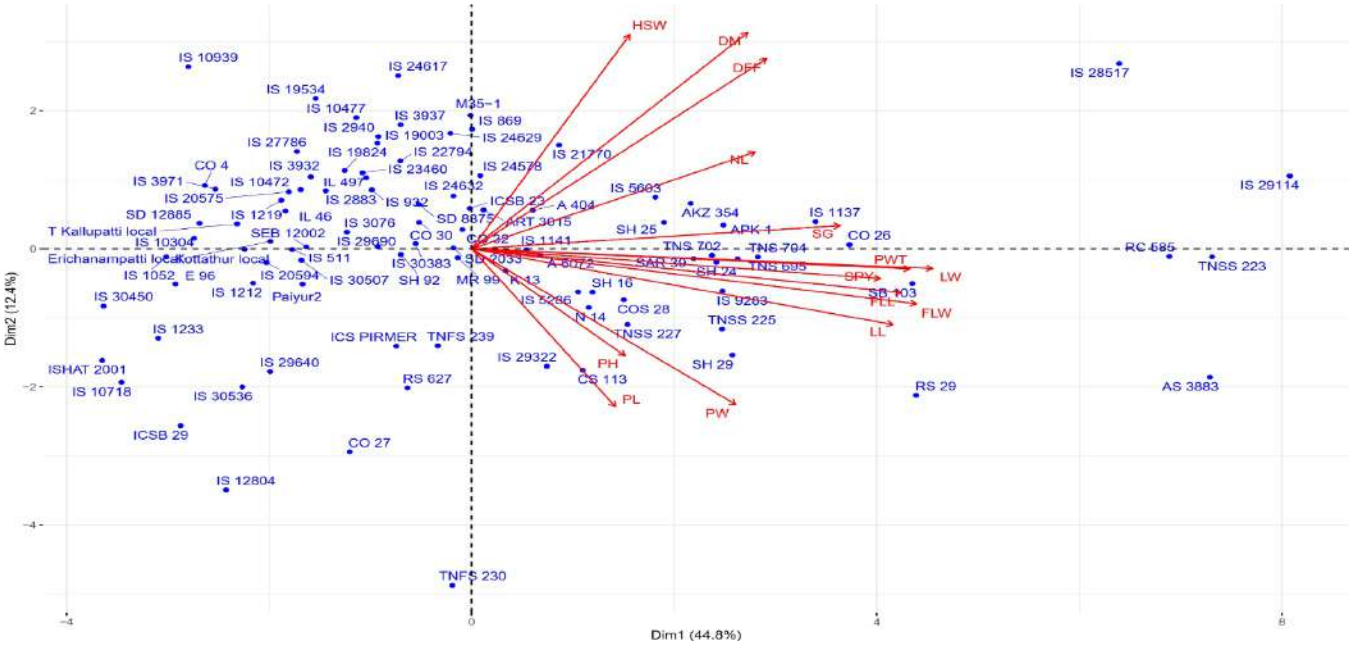

Fig. 3: PCA bi-plot showing distribution of ninety-six sorghum genotypes based on fourteen biometrical traits against two principal components.

observed between hundred-seed weight, days to maturity, days to flowering, and the number of leaves per plant. Conversely, obtuse angles between hundred-seed weight and traits such as panicle length, width, and plant height suggested that larger seed size is linked to shorter, narrower panicles and shorter plants, highlighting potential trade-offs in breeding. The acute angle between panicle length and plant height indicated a positive correlation, suggesting that taller genotypes tended to have longer panicles, supporting higher grain yields, an important factor for selecting high-yield genotypes. Additionally, single-plant yield showed a strong positive correlation with panicle weight, leaf width, flag leaf length, and width, emphasizing that these traits were crucial for increasing productivity in past sorghum breeding efforts.

The PCA biplot (Fig. 3) grouped all the sorghum genotypes according to phenotypic traits represented by the first two principal components (PC1 and PC2), revealing relationships among the genotypes on the basis of yield and yield-contributing traits. The genotypes were mostly distributed across two quadrants, indicating their similarities or differences on the basis of the fourteen traits evaluated. Genotypes such as IS 28517 and IS 29114 in the upper right quadrant exhibited traits such as high hundred-seed weight, extended days to maturity, days to 50% flowering, a greater number of leaves per plant, and thicker stems. Conversely, genotypes such as RS 29 and AS 3883 in the lower right quadrant shared characteristics such as high panicle weight, single plant yield, wider leaves, longer flag leaves, broader flag leaf width, increased leaf length, panicle width, taller plant height, and greater panicle length. This distribution highlights the trait-specific grouping among the sorghum genotypes.

Conclusion

This study assessed the genetic diversity of 96 sorghum genotypes using Mahalanobis's D^2 and principal component analyses, revealing significant variation in yield-related traits. The genotypes were clustered into 10 groups, with specific clusters offering unique traits suited to distinct breeding goals. For example, IS 28517, IS 29114, and TNSS 223 were ideal for biomass production, IS 10939 for high seed weight, AS 3883 for high yield, RS 627 and RC 585 for early maturity and enhanced photosynthesis, respectively. High intercluster distances suggest potential for heterotic hybrids, particularly between distinct genotypes such as IS 10939, AS 3883, and RS 627. PCA highlighted leaf width, panicle weight, plant height, and flag leaf traits as the primary contributors to phenotypic diversity. These findings provide a basis for selecting high-yielding, resilient genotypes, supporting sorghum productivity and sustainability in dryland regions.

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RESEARCH ARTICLE

DUS Characterization of Dahlia (*Dahlia variabilis*)

Priyanka Thakur[1]*, Raghunath Sadhukhan[2], Tapas Choudhary[2], Manish Kumar Srivastava[1] and Simran Kashyap[1]

Abstract

Characterisation and evaluation of native germplasm are fundamental for facilitating genetic improvement, safeguarding varieties, and conserving biodiversity. In this study, thirty-two dahlia (*Dahlia variabilis* L.) genotypes were evaluated over two consecutive years (2022–23) and (2023–24) at the Regional Horticultural Research and Training Station (RHRTS) Dhaulakuan, Sirmour (H.P). Morphological characteristics (51) were used for the identification and grouping of dahlia genotypes. A set of 51 morphological traits was used to distinguish and classify the genotypes in line with the DUS (Distinctiveness, Uniformity, Stability) testing framework developed by RHRTS, Dhaulakuan, Sirmour, in collaboration with BCKV, Kalyani (West Bengal) funded by PPV & FRA, India. The DUS characteristic profile was developed for all 32 genotypes, enabling clear varietal grouping and identification.

Keywords: Characterization, DUS, Dahlia, Genotype

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Introduction

Dahlia (*Dahlia variabilis* L.) is a highly valued tuberous-rooted, perennial and herbaceous flowering plant, celebrated for its stunning and showy blooms. Native to Mexico, it was introduced to India in 1857 by the Agri-Horticultural Society of India (formerly the Royal Agri-Horticultural Society of India). Dahlias are grown for ornamental purposes due to their aesthetic characteristics, which are crucial for the ornamental industry. They are easy to grow both in the ground and pot. Dahlias are extensively used for exhibition, garden display and home decoration and as a cut flower. It is commercially propagated by stem cuttings. Also propagated by seeds, division of tuberous roots and micro-propagation methods. Because of the harsh weather conditions in India during the summer, it is primarily produced as a winter bloom. The Netherlands is a major producer of tuberous-rooted Dahlias, supplying 50 million tubers annually to international markets (Singh *et al.,* 2023; Kumar *et al.,* 2024).

National and international intellectual property authorities such as the UPOV (International Union for the Protection of New Varieties of Plants) and India's PPV&FRA (Protection of Plant Varieties and Farmers' Rights Authority) rely on phenotypic (DUS: Distinctness, Uniformity, Stability) traits as the foundation for registering plant varieties and granting breeders' rights. These morphological characteristics serve not only to

distinguish varieties for legal protection, but also support broader investigations in genetics, taxonomy, evolutionary studies, diversity assessment and their economic utilization. With this in mind, the present research aimed to explore the existing diversity within dahlia (*Dahlia variabilis* L.) germplasm. The study evaluated thirty-two dahlia genotypes using a set of 51 DUS descriptors to assess variability and distinct morphological traits**.**

Materials and Methods

The present study was carried out at the Regional Horticultural Research and Training Station Dhaulakuan, Sirmour, with three replications. Terminal (apical) cuttings were used as a vegetative method for the rapid multiplication of true-to-type plants. Healthy, disease-free tubers were first planted in pots or sand beds under protected conditions. When new shoots emerged and attained a length of 7–10 cm, the terminal portions were selected for cuttings. Terminal cuttings were taken with 2–3 nodes and a few leaves. The lower leaves were removed to reduce transpiration losses. The basal portion of the cutting was treated with rooting hormones such as IBA at 500–1000 ppm to promote early and better root initiation. The media combination of cocopeat + sand was found to be best for root development during Sept-October. Rooted cuttings were transplanted in the fields having a plot size of 1.2 m × 1.2 m with a spacing of 60 cm × 40 cm. Observations were recorded on ten randomly selected plants in each replication as per DUS guidelines. The data on 51 different morphological characters were collected from the selected genotypes (10 plants/genotype) during two consecutive years (2023 and 2024) based on the PPV&FRA - drafted DUS guidelines of dahlia. 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. Flower colour observations were standardized using the Royal Horticultural Society (RHS) colour chart. Colour determinations using a colour chart were made in the middle of the day in a room without direct sunlight because daylight varies. These determinations are made with the plant part placed against a contrasting background. Genotypes studied were Aditya, Agni, Bhikhu's Mother, Black Eternity, Blackout, Chitchor, Cooch Behar, Dust Stone Red, Dust Stone Orange, Eternity, Gargi, Giani Zail Singh, Glory of India, Good Day, Hiranmoyee, Jishu, Kamla, Kenya Blue, Kenya White, Kenya Orange, Kenya Yellow, Kenya Gerua, Lal Bai, Matungini, Minu, Mother Teresa, Piusenia Pink, Piusenia White, Provujee, SP Kamla, Shubhra and Tenzing Norgay.

Results and Discussion

Vegetative characters

Anthocyanin pigmentation, growth habit, stem girth: Vegetative traits utilized for the characterization of the germplasm, along with the number of genotypes identified for each trait, are presented in Table 1. A

Table 1: Genotypes identified for each characteristic state in the studied dahlia germplasm

Sr. No.	Characteristic	State and No. of genotypes
1	Leaf Anthocyanin	Absent (28) Present (4)
2	Bud Anthocyanin	Absent (29) Present (3)
3	Position of terminal bud on stem	Straight (20) Curved (12)
4	Plant growth habit	Upright (27) Spreading (5)
5	Plant Height (cm)	Very short (<50) (2) Short ⩾51-70) (8) Medium (⩾ 70-90) (9) Tall (⩾ 90-110) (6) Very tall (>110) (7)
6	Stem anthocyanin	Absent (26) Present (6)
7	Stem colour	Yellow Green 145B (2) Greyed Orange 177A (7) Greyed Purple 187A (23)
8	Stem Girth (mm)	Thin (<9) (3) Medium (⩾ 9-14) (19) Thick (>14) (10)
9	Leaf Type	Simple (4) Pinnate (27) Bipinnate (1)
10	Leaf Wing	Absent (19) Present (13)
11	Leaf Texture	Hard (17) Soft (15)
12	Leaf glossiness	Absent (24) Present (8)
13	Leaf pubescence	Absent (23) Present (9)
14	Leaf Length (cm)	Small (<10) (2) Medium (⩾10 -20) (20) Large (>20) (10)
15	Leaf Width (cm)	Narrow (<10) (2) Medium (⩾10 -20) (28)

		Broad (>20) (2)
16	Leaf colour RHS Colour charts	Yellow Green 146A (5) Green 137A (24) Purple Green NN137A (3)
17	Leaf Tip	Pointed (12) Tapering (9) Triangular (10) Elongated (1)
18	Leaf Shape	Ovate (14) Elliptic (16) Oblanceolate (2)
19	Leaf vein	Depressed (10) Flat (10) Raised (12)
20	Leaflet shape of base	Acute (10) Obtuse (10) Rounded (1) Asymmetric (3) Truncate (3) Cordate (0)
21	Peduncle length (cm)	Short (<20) (6) Medium (⩾20-40) (20) Long (>40) (7)
22	Anthocyanin of peduncle	Absent (21) Present (11)
23	Length of peduncle above leaf node (cm)	Short (<10) (6) Medium (⩾10-15) (21) Long (>15) (5)
24	Flower heads: position in relation to foliage	Below foliage (1) At same level (3) Above foliage (28)
25	Flower head attitude	Drooping (5) Horizontal (5) Upright (22)
26	Flower head length (cm)	Short (<5) (4) Medium (⩾5- 10) (21) Long (> 10) (7)
27	Flower head diameter (cm)	Miniature (< 10) (2) Small (⩾10- 15) (5) Medium (⩾15-17) (16) Large (⩾17- 20 cm) (4) Dinner - plate sized (>20 cm) (5)
28	Flower head type	Single (1) Semi double (1) Daisy eyed (2) Decorative (14) Pompon (12) Cactus (2)
29	Flower collar segments	Absent (29) Present (3)
30	Flower Disc	Absent (29) Present (3)
31	Ray floret: upper surface	Smooth (16) Keeled (16)
32	Ray florets: No. of keels	One (2) Two (28) More than two (2)
33	Ray floret: twisting	Absent (28) Present (4)
34	Ray floret: shape of apex	Pointed (23) Dentate (4) Rounded (2) Tapering (1) Dome (1) Twisted (1)
35	Length of Ray florets(cm)	Short (<5) (7) Medium (⩾5- 10) (16) Long (>10) (9)
36	Width of Ray florets (cm)	Narrow (<3) (7) Medium (⩾3- 5) (18) Broad (>5 (7)
37	Ray florets: Longitudinal axis	Incurving (25) Straight (4) Reflexing (3)
38	Ray floret: number of colours	Single (25) Bicoloured (4) Multicoloured (3)
39	Ray Floret: Colour of lower surface	Same (25) Different (7)
40	Flower Colour: Ray floret primary colour (RHS colour charts)	White 155 (4) Yellow 3 (6) Orange 25 (5) Red N45 (8) Red Purple 59 (7) Purple NN 78 (2)
41	Distribution of second colour of ray florets	At Tips (6) At basal (1) At margins (1) Blended (24)
42	Ray floret: profile in cross section at mid-point	Flat (1) Concave (26) Convex (5)
43	Ray floret: Position of twisting	Apical (6) Middle (22) Basal (5)
44	Anthocyanin on Epicalyx	Absent (22) Present (10)
45	Shape of Epicalyx	Rounded (5) Elongated (27)
46	Flowering period (No. of days after transplanting)	Early (11) Mid (12) Late (9)
47	Duration of flowering (days)	Short (4) Medium (14) Long (14)
48	Vase life (days)	Short (<4) (20) Medium (⩾4- 6) (7) Long (>6) (5)
49	Shape of tubers	Rounded (8) Elongated (24)

considerable degree of variability was observed among the genotypes for most of the evaluated parameters. Anthocyanin pigmentation in the leaf and bud was absent in approximately 98% of the genotypes, with the exception of 'Black Eternity' and 'Tenzing Norgay', where its presence was recorded. The terminal bud was predominantly straight across genotypes, except in 'Tenzing Norgay', which exhibited a curved bud. Growth habit varied from upright to spreading, with the majority of genotypes displaying an upright growth pattern. Plant height ranged from very short to short and medium to tall categories. The overall plant architecture, determined by growth type, habit, and height, plays a crucial role in optimizing resource acquisition from the environment. Most genotypes were categorized as medium to tall in height, except for 'Black Eternity' and 'Minu', which exhibited shorter stature. Kumar *et al.,* (2022) and Thakur *et al.,* (2022) have previously documented such discrepancies, emphasizing the intricate interplay between genetic composition and environmental factors in influencing plant characteristics.

Stem girth classified the cultivars into three categories: thin (<9 mm), medium (9–14 mm) and thick (>14 mm). Most genotypes exhibited medium stem thickness, except for 'Dust Stone Red', 'Dust Stone Orange', 'Matungini' and 'Minu'. Variability in stem girth can be attributed to both genetic differences among cultivars and environmental influences, as also reported by Mounika and Saravanan (2019). Leaf types ranged from simple to pinnate and bipinnate, with the majority of genotypes exhibiting bipinnate leaves and lacking leaf wings. Leaf glossiness and pubescence were largely absent, and most genotypes displayed hard leaf texture. Leaf length ranged from small (<10 cm) to medium (10–20 cm) and large (>20 cm), with most genotypes falling in the medium category for both length and width. These traits collectively reflect the extensive morphological diversity characteristic of dahlia. Leaf colour, assessed using the RHS colour chart, was predominantly yellow-green (146A), while 'Black Eternity' exhibited a distinct purple-green (137A) hue.

Leaf tips were categorized as pointed, tapering, triangular and elongated. Leaf shape varied among genotypes, ranging from ovate to elliptic and oblanceolate, with the majority of genotypes exhibiting an elliptic shape, an acute leaflet base and a triangular tip. Peduncle length ranged from short to long, with medium-length peduncles being most prevalent among the genotypes. Anthocyanin pigmentation was absent in most of the genotypes studied, except for 'Tenzing Norgay' and 'Black Eternity', where it was distinctly present.

Flowering characteristics

Flower head position, flower diameter, colour of florets: Flower heads exhibited upright posture and flower head position above foliage in maximum genotypes. Flower head length was predominantly medium (5–10 cm) across genotypes, consistent with standard dahlia phenotyping protocols. Flower diameter ranged from miniature (>10–15 cm), small (⩾ 10–15 cm), medium (⩾ 15–17 cm), large (⩾ 17–20 cm) to dinner-plate size (>20 cm). Miniature-sized flower heads were observed in genotypes such as 'Dust Stone Red', 'Dust Stone Orange', and 'Mother Teresa', whereas 'Kenya Blue', 'Kenya White', 'Kenya Gerua', and 'Matungini' were classified as dinner-plate types.

Among the various dahlia genotypes, flower types ranged from single, semi-double, daisy-eyed double and double to decorative, pompon and cactus forms. The majority of genotypes exhibited the decorative flower type, while single-flowered types were recorded in 'Agni', and semi-double flowers were observed in 'Lal Bai'. In most genotypes, the flower collar and disc were absent, except in a few genotypes such as 'Cooch Behar', 'Kamla', and 'Glory of India'. In approximately 95% of genotypes, ray florets exhibited two keels. More than two keels were recorded in 'Matungini' and 'Giani Zail Singh', whereas a single keel was noted in 'Piusenia Pink' and 'Piusenia White'. The apex of ray florets was generally pointed, and twisting was absent in the majority of genotypes. Ray floret length varied from short (<5 cm) to medium (⩾ 5–10 cm) and long (>10 cm), with most genotypes exhibiting medium length and width, along with an incurving longitudinal axis. Most dahlia genotypes produced flowers with uniformly coloured ray florets, except for 'Good Day', which exhibited multicoloured florets. Bicoloured ray florets were observed in genotypes such as 'Bhikhu's Mother', 'Gargi', 'Giani Zail Singh', 'Lal Bai', and 'Mother Teresa'.

In all genotypes, the distribution of the second colour on ray florets was blended, with a concave profile observed in cross-section at the midpoint. Flower colour is a key ornamental trait in dahlia, and six distinct flower colour groups were identified among the studied genotypes, following DUS (Distinctness, Uniformity, and Stability) characterization guidelines. The position of ray florets varied among apical, middle, and basal regions, with approximately 80% of genotypes displaying florets positioned at the middle. Anthocyanin pigmentation in the epicalyx was absent in most genotypes, and an elongated epicalyx shape

was observed in 90% of them. Flowering period was categorized into early (<70 days), mid (⩾ 70–100 days), and late (>100 days). The majority of genotypes fell under the early and mid-flowering categories, while only a few exhibited a short flowering duration. Vase life was generally short in most genotypes, though it varied significantly among cultivars, likely due to differences in carbohydrate accumulation. Similar results were also recorded by Kumar *et al.,* (2021). Regarding tuber morphology, 90% of the genotypes possessed elongated tubers, while a few exhibited round-shaped tubers.

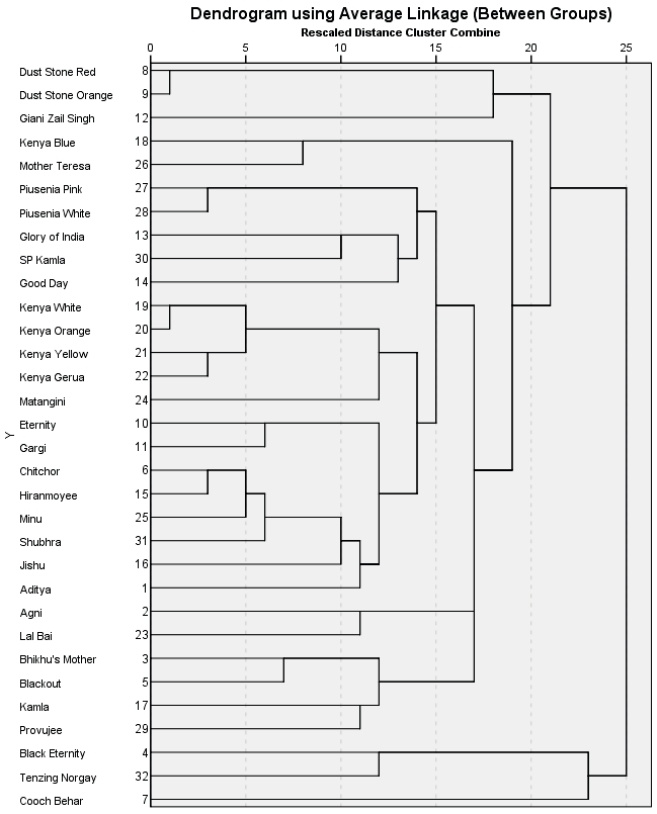

Fig. 1: Hierarchical cluster analysis dendrogram of dahlia genotypes based on morphological data using average linkage (between groups).

Conclusion

The present study revealed the vast variability within characterized dahlia genotypes for different vegetative and flowering traits. Characterized data of different traits could be used as a reference collection for the identification of dahlia varieties. Hierarchical cluster analysis separated the genotypes into different clusters (Fig. 1). The information on different traits is also helpful for future improvement programmes for developing novel type varieties. The given information will also help the breeders and nurserymen to seek the protection of their new material under PPV and FRA, New Delhi.

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SHORT COMMUNICATION

Distinctiveness, Uniformity and Stability of Quantitative Characters of Traditional Rice (*Oryza sativa* L.) Cultivars of West Bengal

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Abstract

The traditional cultivars need to be collected, conserved, and characterized to identify the desirable characters for use in rice improvement programmes. In the present study, 38 traditional rice cultivars were characterized based on 18 quantitative traits, following the guidelines of the Protection of Plant Varieties and Farmers' Rights Act (PPV&FRA) for rice. In addition to those 18 characters, the yield potential of the traditional cultivars was also recorded. Quantitative characters showed highly significant variations among the cultivars. The cultivars were classified into different categories for each quantitative character. Many cultivars were identified with desirable trait(s) along with good yield potential. KNS-2-D-3-1, KNS-2-D-5-1, KNS-2-D-11, KNS-2-D-5 and Kataribhog had high yield potential with many other advantageous traits. This study will be beneficial for breeders in the selection of desirable cultivars for a specific trait. Utilizing this data, different cultivars can be protected under PPV&FRA (2001) and for the farmers, the outcome of research will be helpful to select the appropriate traditional cultivars that possess multiple desirable characters for direct cultivation. Based on this DUS characterization, 10 traditional cultivars, namely Biru, Lal Bhog, Desi Pizam, Dhariyal, Dungru, Gujri Nunia, Kaishali, Lal Khaiyam, Lal Manshra, Loha Swarna, Mala Sundari, Nageswari, Sugandhi Dhepi, and Swet Nunia, have been submitted to PPV&FRA for registration.

Keywords: Amylose content, DUS characterization, Grain shape, Traditional rice

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Introduction

The security of the long-standing practice of cultivation of traditional cultivars, which may have enormous potential for a donor of key traits, is now being threatened by the introduction of high-yielding varieties and improved cultivation practices. The Government of India enacted the Protection of Plant Varieties and Farmers' Rights Act, 2001 (PPV&FRA), to protect plant cultivars based on the Distinctiveness, Uniformity, and Stability (DUS) test. This unique and model Act treats farmers and breeders as partners in their efforts for sustainable food security. A number of processes are involved in the process of identifying a cultivar, including verification that the cultivar is unique from all others that are known to exist, purity of the cultivar, and characterization that lists all of its characteristics. For a cultivar to be classified as a unique entity, the concepts of distinctness, uniformity, and stability are essential. Many rice researchers used this guideline for characterization of the landraces (Surje *et al*., 2022; Akshay *et al*., 2022; Deepika and Devaraju, 2023; Shai Prasanna *et al*., 2024; Ivin Subakar and

Anbuselvam, 2025), conservation through registration under PPV&FRA (Surje *et al*., 2022; Roy *et al*., 2022) and utilization of the characterized germplasm in rice improvement (Roy *et al*., 2022; Roy *et al*., 2025).

The country has a diverse range of indigenous cultivars and landraces farmed throughout various states. According to Shobha Rani and Krishnaiah (2001), each state has unique aromatic rice cultivars that thrive in its region. Improving these rice cultivars is often neglected due to their low export value. Various approaches are needed to establish the identity and distinctiveness of rice cultivars produced in India, particularly in North-eastern India (Talukdar *et al*., 2012; Chakravorty and Ghosh, 2013; Surje *et al*., 2022). Landraces and wild species have useful traits that can be used in breeding to create high-yielding rice cultivars that are resistant to biotic and abiotic challenges (Tirkey *et al*., 2013). Notably, most of the traditional cultivars are photoperiod-sensitive.

Modern rice cultivars are historically generated through selection, hybridization, and backcrossing with regionally adapted, high-yielding genotypes. The restricted number of paternal lines results in a narrow genetic base. Crops with high genetic uniformity may be more susceptible to epidemics. The genetic diversity of rice in our country is a key source for introducing new traits or developing new plant types (Roy *et al*., 2022).

Quantitative phenotypes are often expressed with a wide range of variation. Those characters show continuous variations among the genotypes within a species. Study of numerical variations of genotypes of a species provides exact values of the range of variations, mean of the study materials and to identify the genotypes possessing the desirable values for a particular character in a collected germplasm. A collection has limited practical value unless it is correctly appraised (Chang, 1976). This study aims to define the collected traditional cultivars of rice available at the rice repository of the university and identify key features for varietal development by breeders, and recognise the significance of local landraces. With these objectives, 18 quantitative characters have been studied for the 38 traditional cultivars of rice.

Materials and Methods

Thirty-eight traditional genotypes were used in this study. Twenty-one cultivars were earlier obtained from Shri Sumanta Misra, Pandapara Kalibari village, Jalpaiguri district, West Bengal, one from Manipur and the remaining 16 were the pure lines isolated from Kalonunia (Geographical Indication, the Registration No: 743), a popular traditional aromatic rice of the northern part of West Bengal. All these cultivars are maintained at the Rice Repository, Department of Seed Science and Technology, Uttar Banga Krishi Viswavidyalaya. A list of the 38 genotypes is given in Table 1.

Table 1: List of the traditional rice cultivars used in the study

Sl.No.	Name of Farmers' Cultivars	Collection place/ name of the seed provider
1.	Lal Khayiam	Shri Sumanta Misra
2.	Desi Paizam	Vill & P.O. Pandapara
3.	Sweet Nunia	Kalibari
4.	Biru	Jalpaiguri district
5.	Sweet Paizam	West Bengal
6.	Dalbhog	PIN 735132
7.	Malasundari	
8.	Kaishali Binni	
9.	Simabhog	
10.	Guyri Nunia	
11.	Loha Swarna	
12.	Swarnali	
13.	Binnafuli	
14.	Dhanraaj	
15.	Paddy Queen	
16.	Dungru	
17.	Sugandhi Dhepi	
18.	Kala Khayiam	
19.	Lal Manshar	
20.	Dhariyal	
21.	Nageswari	
22.	Chakhao Amubi	Manipur
23.	KNS-2-D-3	Rice Repository
24.	KNS-2-D-11	Department of Seed
25.	KNS-2-D-12	Science and Technology
26.	KNS-2-D-15	Uttar Banga Krishi
27.	KNS-2-D-9	Viswavidyalaya
28.	KNS-2-D-10	Pundibari, Cooch Behar
29.	KNS-2-2	736165, West Bengal
30.	KNS-2-2/2-3	
31.	KNS-2-1/2-3	
32.	KNS-2-D-5	
33.	KNS-2-D-4	
34.	KNS-2-D-5-1	
35.	KNS-2-D-3-1	
36.	KNS-2-1-6	
37.	KNS 2-3	
38.	Kataribhog	

The experiment was conducted during two consecutive *kharif* seasons in 2022-23. Thirty-day-old seedlings were transplanted by maintaining row-to-row and hill-to-hill distances of 30 cm and 25 cm, respectively. For each cultivar, five rows (length 5.0 m) were transplanted. The standard agronomic practices (Roy, 2023) were followed, compatible with the Terai zone, in order to achieve a good crop stand and subsequently better grain yield. Following transplanting, a 4-6 cm thin layer of water was kept in the field to prevent the growth of weeds. Hand weeding was done as and when required. Pest and disease control was also followed as per requirement.

Observations

Observations on 18 quantitative characters were taken on 10 plants of each cultivar from each replication. Data were recorded following the descriptor of PPV&FRA (2007) on rice- "Guidelines for the Conduct of Test for Distinctiveness, Uniformity, and Stability on Rice". The data were recorded at different stages of plant growth with the appropriate procedures for the following characters. In addition to 18 DUS characters, the yield data of the cultivars were also recorded.

i. **Length of leaf blade (cm):** The length of the leaf blade was determined by measuring it from the leaf collar to the leaf's apex. Leaf length was measured during the launching phase.

ii. **Width of leaf blade (cm):** The width was measured at the widest point of the leaf blade. The leaf breadth was observed during the booting stage.

iii. **Stem thickness (mm):** Stem thickness was measured on some individual stems during the milky stage. Stem thickness was measured using a digital Vernier calliper.

iv. **Days to 50% flowering:** The time of heading was observed when half of the inflorescence emerged from the booting stage, and the observation was conducted through visual appraisal of a single plant group.

v. **Days to maturity:** The time of maturity was observed during the ripening stage, when the terminal spikelets had ripened. The observation was conducted through visual assessment of a specific group of plants.

vi. **Stem length (excluding panicle) (cm):** The stem thickness was measured on several individual stems during the milk development stage.

vii. **Panicle length (cm):** The panicle's length was determined by measuring it from the base to the apex during the ripening stage, when the terminal spikelets ripened.

viii. **Number of panicles/per plant:** The number of panicles per plant was counted during the ripening stage.

ix. **Length of longest awn (cm):** The length of awn was measured individually by a measuring scale during the maturation stage, when the terminal spikelets had ripened.

x. **Test weight (g):** The observation was taken by measuring the weight of 1000 fully developed grains that had been desiccated to 13% moisture content at the caryopsis hard stage, as indicated by the fact that they could no longer be dented by a thumbnail and that over 90% of the spikelets had ripened.

xi. **Grain yield (kg/ha):** Grain yield per plant was recorded for each cultivar, and it was converted to kg/ha.

xii. **Grain dimension:** The grain length (mm) was measured from the base of the lowermost sterile lemma to the apex of the lemma, whichever is longer, measuring the longitudinal dimension from 10 well-developed grains. The grain width (mm) was determined by the distance between the lemma and the palea at the broadest point, or dorsiventral diameter, as measured from 10 grains.

xiii. **Kernel (decorticated grain) length (mm):** The grain length was determined by measuring the longitudinal dimension from the base to the apex of 10 well-developed decorticated grains.

xiv. **Kernel (decorticated grain) width (mm):** The grain width was determined by the dorsiventral diameter, which was measured from 10 decorticated grains and represented the distance across the kernel at the broadest point.

xv. **Decorticated grain shape:** According to Ramaiah (1969), grains were classified into six categories based on their length and L:B ratio: short slender, short bold (SB) (SS), medium slender (MS), long slender (LS), long bold, and extra long slender (L:B). The classification detail is as follows:

Decorticated grain types	Decorticated grain length	L:B ratio
Short slender (SS)	>6 mm	≥3
Short bold (SB)	>6 mm	<2.5
Medium slender (MS)	>6 mm	2.5 - 3.0
Long slender (LS)	≥6 mm	≥3
Long bold (L:B)	≥6 mm	<3.0
Extra-long slender	≥7.5 mm	≥3

xvi. **Presence of amylose in endosperm:** Starch is composed of two components, such as amylose and amylopectin. Amylose is a linear polymer of glucose. The glucose units are joined by α-1-4-glucoside linkages. Amylose exists in a coil for and each coil contains six glucose residues. Iodine is adsorbed within the helical coils of amylose to produce a blue-coloured complex, which is measured colourimetrically. The simplified Juliano (1971) procedure was employed to assess the amylose content.

xvii. **Decorticated grain aroma:** Aroma was assessed following the procedure as outlined by Juliano (1971). Added 15 ml of water to 5 g of rice sample in a test tube (200 mm x 35 mm) and soaked for 10 minutes. The sample was cooked in the water bath for 15 minutes. Then the cooked rice was transferred into a petri dish. The cooked rice was cooled by keeping it in the refrigerator for 20 minutes. Then the petri-plates were opened and the contents were smelled. The samples possessing the scent, as one could easily feel, produce a sharp and readily recognizable aroma and the cultivars were classified as strongly aromatic, mildly aromatic and non-aromatic.

Results and Discussion

All the characters, namely length of leaf blade, width of leaf blade, stem thickness, days to 50% flowering, days to maturity, stem length (excluding panicle), length of main axis of panicle, number of panicles per plant, test weight, grain length, decorticated grain length, amylose content and yield per plant showed high significant variation among the traditional cultivars. However, grain width exhibited non-significant variation among the cultivars, and decorticated grain width had significant variation among the cultivars at 5% level.

Length of leaf blade

Length of leaf blade varied from 25.30 cm to 59.73 cm with a mean of 37.11 cm. Four traditional cultivars were found to have long leaf blades, 30 were classified as medium, and four cultivars were categorized as short (Table 2). Significant variation in the length of the leaf blade was also reported by Subudhi *et al*. (2012), Chakravorty and Ghosh (2012, 2013), Subba Rao *et al*., (2013), Surje *et al*., (2022), and Shai Prasanna *et al*., (2024).

Table 2: Classification of traditional rice cultivars based on different agronomic characters

Sl.No.	Characters	Classes	Number	Frequency (%)
1.	Length of leaf blade	Short (30 cm)	04	10.53
		Medium (30-45 cm)	30	78.95
		Long (45 cm)	04	10.53
2.	Width of leaf blade	Narrow (1.0 cm)	24	63.16
		Medium (1-2 cm)	14	36.84
3.	Stem thickness	Thin (0.40 cm)	20	52.63
		Medium (0.40-0.55 cm)	16	42.11
		Thick (0.55 cm)	02	5.26
4.	Days to 50% flowering	Late (111-130 days)	16	42.11
		Very Late (130 days)	22	57.89
5.	Days to maturity	Late (141-160 days)	26	68.42
		Very Late (160 days)	12	31.58
6.	Stem length (excluding panicle)	Very short (91 cm)	01	2.63
		Short (91-100 cm)	05	13.16
		Medium (111-130 cm)	31	81.58
		Long (131-150 cm)	01	2.63
7.	Panicle length	Medium (21-25 cm)	14	36.84
		Long (25-30 cm)	15	39.47
		Very Long (30 cm)	09	23.68
8.	Number of panicles/plant	Few (11)	03	7.89
		Medium (11-20)	20	52.63
		Many (20)	15	39.47
9.	Length of the longest awn	Very short (⩽ 0.9 cm)	06	31.58
		Short (1.0-1.90 cm)	07	36.84

		Medium (2.0-2.9 cm)	03	15.79
		Very Long (3.90 cm)	03	15.79
10.	Test weight	Very low (15 g)	07	18.42
		Low (15-20 g)	18	47.37
		Medium (21-25 g)	07	18.42
		High (25-30 g)	04	10.53
		Very high (30 g)	02	5.26

Width of leaf blade

The width of the leaf blade varied from 0.30 to 1.73 cm with a mean of 0.91 cm. As per the classification of PPV&FRA (2007), none of the cultivar's leaf width fell under the 'Broad' category (Table 2). Fourteen cultivars were categorized as medium, and 24 were classified as narrow. Earlier reports furthermore confirmed the existence of significant variation among the traditional cultivars of rice in respect of the width of the leaf blade (Subba Rao *et al*., 2013; Surje *et al*., 2022; Shai Prasanna *et al*., 2024).

Stem thickness

Stem thickness varied from 0.18 cm to 0.62 cm with a mean of 0.37 cm. Only two cultivars were found to have 'thick' stems, nine were classified as 'medium', and 27 cultivars were categorized as 'thin' (Table 2). Stem thickness is an important parameter of rice plants to decide the lodging status of cultivars. Taller cultivars need to have a thicker stem to provide lodging tolerance. Many rice researchers (Chakrabarty *et al*., 2012; Chakravorty and Ghosh, 2013; Surje *et al*., 2022) have observed significant variation among the traditional cultivars of rice.

Days to 50% flowering

Days to 50% flowering varied from 126.00 to 137.50 days with a mean of 131.21 days. None of the cultivars fell under the categories, namely 'Very Early', 'Early' and 'Medium'. Sixteen cultivars were classified as 'Late'. The majority of the cultivars used in this experiment were identified as 'very late' (Table 2). Days to 50% is an imperative trait that makes a decision on the duration of the cultivar. For intensive and extensive cultivation, early cultivars are in favorite to increase the cropping intensity. However, in this study, none of the cultivars were categorized under 'Very Early', 'Early' and 'Medium'. Most of the cultivars were 'Late' or 'very late'. These results corroborate the findings of Subba Rao *et al*., (2013), Surje *et al*., (2018) and Surje *et al*., (2022).

Days to maturity

Days to maturity ranged from 148.50 to 164.50 days with a mean of 157.59 days. None of the genotypes categorized under 'Very Early', 'Early' and 'Medium' (Table 2). Twenty-six traditional cultivars were categorized as 'Late', and 12 were found to be 'Very Late'. Our study confirmed that most of the traditional cultivars were 'Late' and 'Very Late'. A similar study was made by Subba Rao *et al*., (2013) and Surje *et al*. (2022).

Stem length (excluding panicle)

Stem length ranged from 50.66 to 100.30 cm with a mean of 78.32 cm. Only one cultivar, Swarnali, was recorded as very short (Table 2), and five genotypes were classified as 'Short'; the majority of the cultivars were categorized under the 'Medium' category. None was categorized under 'Very Long'.

Again, the stem length in equivalence with stem thickness is an important trait that makes a decision on tolerance or susceptibility towards lodging. In general, short to medium plant height is preferred as this makes all the intercultural activities. In addition, short height rice cultivars are usually considered as lodging resistant (Anna Durai *et al*., 2015; Zhang *et al*., 2016; Shah *et al*., 2019). As per the findings of many rice workers, the stem length of most of the traditional cultivars was medium and long (Chakravorty and Ghosh, 2013; Surje *et al*., 2022). However, Subba Rao *et al*., (2013) and Gyathri *et al*., (2023) found that nearly all of the traditional cultivars they studied fell under very short, short and medium categories.

Panicle length

Panicle length varied from 20.43 to 40.60 cm with a mean of 27.38 cm. None of the genotypes observed under the 'Very Short' and 'Short' categories (Table 2). Fourteen cultivars were recorded under 'Medium' class, 15 cultivars were classified as 'Long' and nine cultivars fell under 'Very Long' category.

Panicle length is a desirable yield-attributing character, and a longer panicle is preferred. Many rice workers established a positive correlation of panicle length with grain yield per plant in rice (Singh *et al*., 2006; Senapati *et al*., 2009; Roy, 2010; Rangare *et al*., 2012). Very long panicles were observed for Kalojeera (Subba Rao *et al*., 2013), Tulsimukul, Maitee, and Kagey (Surje *et al*., 2022). The length of the main axis of the panicle of most of the traditional cultivars of rice

possessed medium length (Subba Rao *et al*., 2013; Singh *et al*., 2015; Gyathri *et al*., 2023).

Number of panicles/plant

The number of panicles per plant ranged from 8.50 to 32.00 cm with a mean of 18.75 cm. It is an important yield-attributing character. Fifteen cultivars were classified under 'Many' (Table 2), and the majority of the cultivars were categorized under the 'Medium' category.

Most of the traditional cultivars had more than 11 panicles per plant. It showed that the traditional cultivars of rice have high tillering potential. Similar results were also reported by Chakrabarty *et al*., (2012); Subba Rao *et al*., (2013); and Surje *et al*., (2022). The traditional cultivars that possessed less than 11 panicles per plant were also reported, such as Bahurupi, Bidan Sapru (Subba Rao *et al*., 2013); Dhuladhan, Dahamagra, Sankarsal, Behalsal, Laldhusri, Nagra, Majhisal, Kalma, Sunga Nagra, Asanlaya (Chakravorty and Ghosh, 2012, 2013); Kranti, Heera (PPV&FRA, 2007).

Length of the longest awn

Out of 38 traditional cultivars, 19 were noted to have awn (Table 2). Those 19 cultivars were classified into different classes following the guidelines of PPV&FRA (2007). Six traditional cultivars were categorized as 'Very Short', seven cultivars were categorized as 'Short', three were classified as medium, and another three cultivars were classified as 'Very Long'.

Awn length exhibited continuous variation among the cultivars. Generally, the traditional cultivars bear awns at the tip of the lemma. Variation of the length of the awn was observed by several authors (Joshi *et al*., 2011; Surje *et al*., 2022). Presence of awn is a primitive character of rice and well adapted to adverse environmental factors, *viz*., drought, salinity and low temperature (Chandraratna, 1964).

Test weight

Test weight ranged from 10.50 to 30.30 g with a mean of 19.22 g. Only two cultivars had fallen under the 'Very High' category (Table 2). There were five genotypes under the 'High' category, seven cultivars were categorized under Low', 17 were classified under the 'Low' category, and seven cultivars showed very low test weight.

Test weight is also an important yield-attributing character. This character had a positive and highly significant correlation with grain yield (Senapati *et al*., 2009; Roy, 2010; Rangare *et al*., 2012). Traditional rice cultivars are in generally possess higher test weight. The majority of our study materials had low test weights (15-20 g). These results corroborates the report of Gyathri *et al*., (2023).

Table No. 3: Yield of traditional cultivars

Sr. No.	Cultivars	Grain yield (kg/ha)
1.	Lal Khayiam	4650
2.	Desi Paizam	3750
3.	Sweet Nunia	4500
4.	Biru	5250
5.	Sweet Paizam	7350
6.	Dalbhog	3600
7.	Malasundari	3930
8.	Kaishali Binni	2100
9.	Simabhog	3510
10.	Guyri Nunia	1650
11.	Loha Swarna	4800
12.	Swarnali	1350
13.	Binnafuli	3000
14.	Dhanraaj	4500
15.	Paddy Queen	1350
16.	Dungru	2400
17.	Chakhao Amubi	3000
18.	Sugandhi Dhepi	3630
19.	Kala Khayiam	3900
20.	Lal Manshar	6000
21.	Dhariyal	3690
22.	KNS-2-D-3	4200
23.	KNS-2-D-11	7050
24.	KNS-2-D-12	3750
25.	KNS-2-D-15	3600
26.	KNS-2-D-9	2910
27.	KNS-2-D-10	4290
28.	KNS-2-2	3360
29.	KNS-2-2/2-3	7410
30.	KNS-2-1/2-3	4350
31.	KNS-2-D-5	7380
32.	KNS-2-D-4	4800
33.	KNS-2-D-5-1	4980
34.	KNS-2-D-3-1	5250
35.	KNS-2-1-6	3690
36.	KNS 2-3	2190
37.	Nageswari	5790
38.	Kataribhog	5250
	■ Mean	4162.11
	■ Range	1350-7410

Grain yield

Grain yield ranged from 20.00 to 60.40 g/per plant with a mean of 38.75 g/per plant. The yield per plant had been converted to yield per hectare, considering

150000/plants per hectare. Some of the cultivars showed grain yield more than 6.00 t/ha (Table 3), namely KNS-2-2/2-3 (7410 t/ha), KNS-2-D-5 (7380 t/ha), Sweet Paizam (7350 t/ha), KNS-2-D-11 (7050 t/ha) and Lal Manshar (6000 t/ha). Some of the cultivars that had desirable yield performance were Nageswari (5790 t/ha), Biru (5250 t/ha), KNS-2-D-3-1 (5250 t/ha), Kataribhog (5250 t/ha), KNS-2-D-5-1 (4980 t/ha), Loha Swarna (4800 t/ha), KNS-2-D-4 (4800 t/ha), Lal Khayiam (4650 t/ha), Sweet Nunia (4500 t/ha), Dhanraaj (4500 t/ha), KNS-2-1/2-3 (4350 t/ha), KNS-2-D-10 (4290 t/ha) and KNS-2-D-3 (4200 t/ha).

Some of the cultivars had very low grain yield (Table 3), they were Swarnali (1350 t/ha), Paddy Queen (1350 t/ha), Guyri Nunia (1650 t/ha), Kaishali Binni (2100 t/ha), KNS 2-3 (2190 t/ha), Dungru (2400 t/ha) and KNS-2-D-9 (2910 t/ha).

Grain dimension

Grain length ranged from 5.61 to 10.11mm with a mean of 7.55 mm. None of the cultivars were found under the 'Very Long' and 'Long' categories. Five cultivars were found to be in the 'Medium' group. The majority of the genotypes were categorized under 'Short' (Table 4). Only five cultivars were found in the 'Very Short' category.

Grain width varied from 1.96 to 3.26 mm with a mean of 2.41 mm. Only one cultivar had a grain width less than 2.00 mm and fell under the 'Very Narrow' category (Table 4). The majority of the cultivars had narrow grain width. There were 26 cultivars under the 'Narrow' group. Seven cultivars fall under the 'Medium' category. Only three cultivars were found in the 'Long' grain width class.

Kernel (decorticated grain) length

Decorticated grain length varied from 3.77 to 7.07 mm with a mean of 5.34 mm. As per the classification of PPV&FRA (2007), there are five states, namely 'Short', 'Medium', 'Long', 'Long (for Basmati Type)' and 'Extra Long'. However, Gyathri *et al.,* (2023) have classified them as 'Short (6.0 mm)', 'Medium' (6.1-8.5 mm), 'Long (8.6-10.5 mm)', 'Very Long (10.6-12.5 mm)' and 'Extra Long (12.5 mm)'. Neither of the classifications was felt suitable for our study materials. Therefore, we have classified the rice cultivars as 'Short (6.0 mm)', 'Long (6.0-6.6 mm)', 'Basmati Type (6.61-7.50 mm)', and Extra Long (7.5 mm)', keeping parity with the classification of grain shape under Sl. No. 56 of PPV&FRA (2007). None of the cultivars fell under the 'Extra Long' category (Table 4). Only two cultivars were classified under 'Basmati Type', and four cultivars were classified as 'Long'. The remaining 32 cultivars were classified as 'Short'. A similar study was also carried out by Surje *et al*., (2018).

Decorticated grain width varied from 1.77 to 2.89 mm with a mean of 2.10 mm. Fifteen cultivars showed decorticated grain width less than 2.0 mm, and they were categorized as 'Narrow'. Twenty cultivars were classified as the 'Medium' category (Table 4). There were only three cultivars under the 'Broad' category. A similar result was also noted by Surje *et al*., (2022) and Gyathri *et al*., (2023).

Decorticated grain L:B ratio

The L:B ratio of decorticated grain wide-ranging from 1.92 to 3.45. Two cultivars were categorized under 'Broad' (Table 4), 23 were found to aggregate under 'Medium' class, and 13 cultivars had a low L:B ratio.

Decorticated grain shape

Decorticated grain dimension (decorticated grain length and decorticated grain width) plays an important role in deciding the grain shape (Harberd, 2015; Li *et al*., 2022). Furthermore, the shape of rice grains caters to consumers' preferences. In addition, the long slender grain rice cultivars are preferred by the consumers of the United States of America, Western Europe and most of the Asian countries like India, China, Thailand and Pakistan. Whereas the short-grained rice cultivars are preferred by Japan, South Korea and Sri Lanka (Unnevehr *et al*., 1992). Within the country, preferences differ across states and locations. Urban and suburban people prefer slender rice, while bold rice is preferred by rural people of Kerala. For any special celebration, long, slender and extra-long slender grain is preferred. Considering the preference for decorticated grain shape, the cultivars under this study were classified as short slender, short bold, medium slender, long slender, long bold, Basmati type and extra-long slender.

None of the cultivars was found under 'Extra Long Slender' and 'Basmati Type' categories (Table 4). Fifteen cultivars were medium slender; 16 cultivars were short, bold, and only one cultivar was short, slender. The largest part of the cultivars fell under 'Short Bold' and 'Medium Slender' categories. Study of Surje *et al*., (2018) also showed a high number of cultivars had 'short bold' decorticated grains. Chakrabarty *et al*. (2012) reported predominance of 'Long Bold' and 'Extra Long' decorticated grains. However, in the study materials of Surje *et al*., (2022), the majority of the cultivars were 'Long Bold'. In the study materials of Gyathri *et al*., (2023), the greater part of the cultivars were under the 'Long Bold' category.

Presence of amylose in endosperm

All the cultivars were found to have amylose in their endosperm. Amylose content determines the eating quality of cooked rice. It is positively correlated with hardiness and negatively correlated with stickiness (Windham *et al*., 1997). Amylose content has long been used as a parameter for promotion of rice cultivars in AICRIP trials in India. According to PPV&FRA (2007), it has three states- low (10-19%), medium (20-25%), high (26-30%) and very high (>30%). The waxy rice contains < 5%.

In our study, the amylose content ranged from 20.81-27.89% with a mean of 23.97%. None of the cultivars fell under 'Low' and 'Very High' categories (Table 4). Most of the traditional rice cultivars in this study fell under the 'Medium' class. Thirty-four cultivars were found to have amylose content of 20-25%. This result corroborates the findings of Surje *et al*., (2018). This is the recommended range for Indian consumers. Under this range, cooked rice will be non-sticky, which is preferred by Indian consumers. Only four cultivars (10.53%) had amylose content in the higher range (26-30%).

Amylose content is important because firmness and stickiness are two parameters of cooked rice that influence the consumers' preferences. Waxy rice has zero or very low (< 5%) amylose content and is often referred to as sticky rice. Japonica rice tends to be low amylose, Tropical Japonica (or Javanica) tends to be intermediate or high, and Indica rice falls into all the amylose classes.

Decorticated grain aroma

The aroma of cooked rice was assessed following the method as explained by Juliano (1971). This character was found trimorphic- strong aromatic, mild aromatic and non-aromatic (Table 4), and the frequency distribution of this character was 63.16%, 21.05% and 15.79%.

Out of the 38 cultivars, 24 were recorded as having a strong aroma, eight were mildly aromatic, and six were non-aromatic. The result indicated that the majority of the cultivars were aromatic. In contrast to the collections of Akshay *et al*., (2022) and Gayathri *et al.,*

Table 4: Classification of traditional rice cultivars based on different grain characters and presence of amylose in endosperm

Sl.No.	Characters	Classes	Number	Frequency (%)
1.	Grain length	Very short (6.0 mm)	05	13.16
		Short (6.1-8.5 mm)	28	73.68
		Medium (8.6-10.5 mm)	05	13.16
2.	Grain width	Very narrow (2.0 mm)	01	2.63
		Narrow (2.1-2.5 mm)	26	68.42
		Medium (2.6-3.0 mm)	08	21.05
		Long (3.1-3.5 mm)	03	7.89
3.	Decorticated grain length	Short (6.0 mm)	32	84.21
		Long (6.0-6.6 mm)	04	10.53
		Basmati type (6.61-7.5 mm)	02	5.26
4.	Decorticated grain width	Narrow (2.0 mm)	15	39.47
		Medium (2.0-2.5 mm)	20	52.63
		Broad (2.5 mm)	03	7.89
5.	Decorticated grain L:B ratio	2.5	13	34.21
		2.5-3.0	23	60.53
		3.0	02	5.26
6.	Decorticated grain shape	Short slender	01	2.63
		Short bold	16	42.11
		Medium slender	15	39.47
		Long slender	01	2.63
		Long bold	05	13.16
7.	Presence of amylose in endosperm	Medium (20-25%)	34	89.47
		High (26-30%)	04	10.53
8.	Aroma	Strong aromatic	24	63.16
		Mild aromatic	08	21.05
		Non-aromatic	06	15.79

(2023), the majority were non-aromatic. In another study by Surje *et al*., (2022), they reported 25 strongly aromatic out of 132 traditional rice cultivars. Gayathri *et al*., (2023) reported only three aromatic cultivars out of 36 genotypes they studied. Many traditional rice cultivars are grown traditionally, which do extremely well in aroma, grain quality and cooking suitability. Huge numbers of aromatic traditional cultivars are available in our country, particularly north and North-eastern states, which are at par with Basmati. Those aromatic traditional rice cultivars may be popularized to improve the farmers' income.

Promising farmers' cultivars

The traditional rice cultivars undertaken in this study registered a wide range of distinctiveness for all the quantitative characters. Similar frequencies for quantitative characters have been reported by several rice workers (Sinha and Mishra, 2013; Kalyan *et al*., 2017; Umarani *et al*., 2017; Manjunatha *et al*., 2018; Gour *et al*., 2019; Priyanga *et al*., 2020; Singh *et al*., 2021; Lavanya *et al*., 2021; Surje *et al*., 2022; Akshay *et al*., 2022).

To find out the better performing cultivars based on yield attributing characters and a few desirable qualitative characters, 10 better performing cultivars were listed in Table 5 for seven yield attributing characters and three quality characters. KNS-2-D-3-1 was found to be the best based on high yield potentiality (5250 t/ha), high number of panicle per plant (24.00 panicle/per plant), comparatively low days to 50% flowering (129.5 days), low days to maturity (155 days), medium amylose content (23.83%) and having medium slender grains with strong aroma.

The next better cultivar was KNS-2-D-5-1 (Table 5). It was recorded as having high yield potential (4980 t/ha), a high number of panicles per plant (29.5 panicles/plant), taking comparatively fewer days to attain 50% flowering (155.5 days), medium amylose content (22.32%) and having medium slender grains with strong aroma.

KNS-2-D-11 had recorded as a high grain yielder (7050 kg/ha), a high number of panicles per plant (23.5 panicles/plant), comparatively low days to 50% flowering (130.0 days), medium amylose content (22.92%) and having strong aroma with short bold grains.

The cultivar, KNS-2-2/2-3, had been reported as a high grain yielder (7050 kg/ha), possessing the maximum number of panicles per plant (32.0 panicles/plant) and having medium slender grains with

Table 5: Character-wise ten best performing cultivars

Characters	Cultivars
Days to 50% flowering	Desi Paizam, Malasundari, Swarnali, KNS-2-D-9, KNS-2-2. KNS 2-3, Dungru, KNS-2-D-3, KNS-2-D-5-1, KNS-2-D-3-1
Days to maturity	Chakhao Amubi, KNS-2-D-9, Desi Paizam, KNS-2-D-3, KNS 2-3, Malasundari, Swarnali, KNS-2-1-6, KNS-2-D-11, KNS-2-D-4
Plant height	Swarnali, Malasundari, Lal Manshar, KNS-2-D-15, KNS-2-D-12, KNS-2-D-10, KNS-2-2, Guyri Nunia, Dhariyal, Sweet Nunia
No. of panicles per plant	KNS-2-2/2-3, KNS-2-D-3, KNS-2-D-10, KNS-2-D-5-1, Lal Manshar, KNS-2-D-15, Biru, Sweet Nunia, KNS-2-D-3-1, KNS-2-D-11
Panicle length	Sweet Nunia, Biru, KNS-2-D-12, KNS-2-D-15, Simabhog, Guyri Nunia, Dalbhog, KNS-2-D-5, KNS-2-D-3, KNS 2-3
Test weight	Sugandhi Dhepi, Chakhao Amubi, Lal Khayiam, Loha Swarna, Dhariyal, Dungru, Swarnali, Malasundari, KNS-2-2
Grain yield	KNS-2-2/2-3, KNS-2-D-5, Sweet Paizam, KNS-2-D-11, Lal Manshar, Nageswari, Biru, KNS-2-D-3-1, Kataribhog, KNS-2-D-5-1
Grain type	Malasundari, Biru, Sweet Paizam, Dalbhog, Kala Khayiam, KNS-2-D-12, KNS-2-D-15, KNS-2-D-9, KNS-2-D-10, KNS-2-2, KNS-2-2/2-3, KNS-2-1/2-3, KNS-2-D-4, KNS-2-D-5-1, KNS-2-D-3-1, Katarybhog
Amylose content	Lal Khayiam, Sweet Nunia, Biru, Sweet Paizam, Dalbhog, Malasundari, Kaishalibinni, Simabhog, Loha Swarna, Dhanraaj, Paddy Queen, Dungru, Chakhaoamubi, Sugandhi Dhepi, Kala Khayiam, Lal Manshar, Dhariyal, KNS-2-D-3, KNS-2-D-11, KNS-2-D-12, KNS-2-D-15, KNS-2-D-9, KNS-2-D-10, KNS-2-2, KNS-2-2/2-3, KNS-2-1/2-3, KNS-2-D-5, KNS-2-D-4, KNS-2-D-5-1, KNS-2-D-3-1, KNS-2-1-6, KNS-2-3, Nageswari, Kataribhog
Aroma	Lal Khayiam, Biru, Sweet Paizam, Kaishali Binni, Sugandhi Dhepi, KNS-2-1/2-3, Nageswari, Kataribhog, Simabhog, KNS-2-D-11, KNS 2-3, Chakhao Amubi, KNS-2-D-3, KNS-2-D-12, KNS-2-D-15, KNS-2-D-9, KNS-2-D-10, KNS-2-2, KNS-2-2/2-3, KNS-2-D-5, KNS-2-D-4, KNS-2-D-5-1, KNS-2-D-3-1, KNS-2-1-6

strong aroma. KNS-2-D-5 also had high yield potential (7380 kg/ha), long panicle (30.86 cm), medium amylose content (24.73%) and short bold grains with strong aroma.

Katarybhog showed high grain yield potential (5250 kg/ha), medium amylose content (23.22%) and possessed a medium slender grain type with strong aroma. This cultivar is local to the Dakshin Dinajpur district of West Bengal. However, it can be cultivated in other northern parts of West Bengal. As documented by NBPGR, it also has a low glycemic acid index (45.72%) [IC0640647; INGR21113].

Apart from the above-mentioned cultivars, Sweet Paizam (7350 kg/ha), Lal Manshar (6000 kg/ha), Nageswari (5790 kg/ha) and Biru (5250 kg/ha) exhibited high grain yield potential and had medium amylose content. Those traditional rice cultivars can be recommended for cultivation. Sweet Pizam and Biru possessed medium slender decorticated grains.

Conclusion

All the quantitative characters, namely length of leaf blade, width of leaf blade, stem thickness, days to 50% flowering, days to maturity, stem length (excluding panicle), length of main axis of panicle, number of panicles per plant, test weight, grain length, decorticated grain length, amylose content and yield per plant showed high significant variation among the traditional cultivars except grain width that had insignificant variation. Some of the cultivars that performed at par with the released high-yielding cultivars, viz. KNS-2-D-3-1, KNS-2-D-5-1, KNS-2-D-11, KNS-2-2/2-3, KNS-2-D-5 and Kataribhog can be recommended for cultivation. Quantitative characters showed highly significant variations among the genotypes. This study will be useful for breeders, researchers and farmers to identify and restore beneficial genes for crop improvement.

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Plant Germplasm Registration Notice*

A total of 180 proposals were received online for the consideration of registration of germplasm for unique/superior traits. The applications were thoroughly screened by the member-secretary, Plant Germplasm Registration Committee (PGRC) and 94 proposals complete in all respects were reviewed by the experts and were presented in the 50th meeting of the PGRC held in virtual mode on June 12, 2023. 67 proposals with unique/novel features belonging to 29 species were recommended for registration by PGRC. The information on registered germplasm is published with the purpose to disseminate the information to respective crop breeders for utilization of these genetic stocks in their crop improvement programmes. Description of the 34 out of the 67 germplasm lines registered after recommendation by PGRC is given below:

1. Meghalaya Lakang (IC648583; INGR23001), a rice germplasm with combined resistance to leaf blast and neck blast

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Among the biotic stresses, blast is one of the most devastating diseases in rice which infects most of the plant parts and leads to cause huge economic losses in term of yield and quality. Identification and development of resistant genotype/sources for blast diseases is most sustainable strategies and widely adopted by the rice breeders to tackle this disease. Rice landraces and its wild relatives which are distributed in North East India are the most potential and unexplored/unexploited sources for tolerance/resistant against the major biotic and abiotic stresses of rice that can be utilized in rice breeding programme for development of resistant varieties. Meghalaya Lakang is a unique landrace adapted to the specific ecology of North Eastern India. This landrace was collected through an exploration trip by scientists of ICAR RC Manipur Centre. After its collection, the germplasm line was purified, maintained and characterized at ICAR RC Manipur Centre, Lamphelpat, Imphal (Table 1). The line was constantly screened for two seasons under Uniform blast nursery (UBN) and natural epiphytic conditions (Two season at ICAR RC Manipur Centre Lamphelpat)

Table 1: Characteristics of Meghalaya Lakang

Plant type	**Tall (125-130 cm)**
Habitat	Cultivated, annual
Days to 50% flowering	95 days
Days to maturity	140-145 days (Late)
Effective tillers	6-8
Grain type	Medium bold/Short bold
Yield per ha	1.8t/ha
Adaptation	Specific environment of Meghalaya and other parts of the North East Hill region
Unique characterizes	Highly resistant to leaf and neck blast, possesses novel gene(s)/ QTL(s)

Edited by: **Anju Mahendru Singh and **Anjali Kak**, Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012, India.*

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and recorded complete resistance to blast. It exhibited combined resistance to leaf blast (score 2 on SES scale) and neck blast (score 1 on SES scale). It was subjected to gene profiling using well known 12 major blast resistance gene (*Pitp, Pi33, Pi54, Pib, Pi20, Pi38, Pita2, Pi1, Piz, Pi9, Pizt* and *Pi40*) and intriguing, they didn't habour any functional allele/positive alleles of the 12 blast resistance genes (Bangale *et al.,* 2017). These revealed that this germplasm contains some unique and novel gene (s)/QTL (s) for blast resistance leaf and neck blast.

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2. RP6253-MV2 (IC648978; INGR23002), a rice germplasm with high nitrogen use efficiency (NUE) under N-Low and N-50 input

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In the studies of genetic improvement for nitrogen use efficiency (NUE) in rice (*Oryza sativa*), RP6253-MV2 is identified as promising cultivar under low nitrogen (N) field conditions (without external N application) and 50% of the recommended N (N-50) expressed in terms of grain yield. RP6253-MV2 was developed from the Varadhan × MTU1010 cross combination. It has 88-90 days to 50% flowering and possesses long bold (LB) grain type (Table 1). It was evaluated in the AICRIP plant physiology RNUE trial (Evaluation of Radiation and Nitrogen use efficient promising rice genotypes) during 2015-18 (IIRR Progress Report, 2018). The overall mean grain yield was noted as 557 kg/m^2, 510 kg/m^2, 398 kg/m^2 in N-100, N-50 and N-Low treatments. In addition, RP6253-MV2 has the following significant features: i). 17%, 16% and 21% yield superiority over Varadhan (Positive check) in the recommended dose of N (N-100), 50% of the recommended dose of N (N50) and low N treatments during 2017 and 2018. ii). The per cent reduction in grain yield under N-50 treatment is on par with Varadhan. iii). In low N treatment, 40% reduction was observed in RP6253-MV2, while Varadhan exhibited 45% reduction in grain yield. RP6253-MV2 was noted at 1445 kg/m^2, 1295 kg/m^2 total dry matter in N-100 and N-50 treatments. The per cent reduction in total dry matter under N-50 treatment is 11%, which is on par with Varadhan. In addition, the AMMI biplot analysis of grain yield under Low-N indicates RP6253-MV2 is the least affected genotype upon low nitrogen treatments (Subrahmanyam *et al.,* 2019). RP6253-MV2, therefore, can be used as a potential donor in breeding rice (*Oryza sativa* L.) for high NUE.

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3. MSM-3 (IC648592; INGR23003), a rice germplasm with increased root length and root volume and better seedling vigour index

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Considering the importance of well-developed root systems for plant growth and yield, a total of 10,500 BPT-5204 seeds were treated with EMS and developed a Samba Mahsuri mutant population in collaboration with CSIR-CCMB. After several series of screening of the mutant population against deep root system and high seed vigour index, a mutant line (TI- 3) was identified, which has higher root length (cm), volume (cm^3), root dry weight (g) as compared to BPT 5204, CR Dhan 201 and MAS 946-1 under aerobic conditions. TI-3 also recorded a higher seedling vigour index as calculated considering the seedling length (SVI-I) and dry weight (SVI-II) (Addanki *et al*., 2018). The selection of TI-3 was done in M2 generation and advanced to M8 through panicle to row method to check the inheritance & stability of the trait. TI-3 showed higher root length (cm) and volume (cm^3), and root dry weight. In addition to these, the line also showed a higher seedling vigour index (SVI- I & II) than wild type (BPT 5204) measured at 14 and 21 days after germination (IIRR, Newsletter-2020). Owing to the above superior traits, the line TI-3 can be deployed in aerobic rice (*Oryza sativa* L.) breeding programs (Table 1). Genotyping of TI-3 was also carried out with 51 SSR markers. Genotypic data revealed that TI-3 had a genetic similarity of 94.12% with that of wild type (BPT 5204).

References

Addanki KR, D Balakrishnan, YV Rao, S Malathi, M Sukumar, B Kavitha and N Sarla (2018) Swarna × *Oryza nivara* introgression lines: A resource for seedling vigour traits in rice. *Plant Genet. Resour.* 17(1): 1-12.

IIRR Newsletter (2020) Vol: 18(2): April- June.

Table 1. Meteorological data from different locations used in the study

S.No	Characteristics	Aerobic	Irrigated	BPT5204 (Irrigated)
1	Days to 50% flowering	99	106	119
2	Number of panicles (No.)	15	16	16
3	Panicle length (cm)	22.6	23.0	18.4
4	Yield/plant (g)	29.4	32.8	22.5
5	Grains per panicle (No.)	210.6	318.7	190
6	Root length (cm)	36.6	33.4	27.52
7	Shoot dry weight (g)	31.8	27.1	19.4
8	Root dry weight (g)	15.7	12.7	6.14
9	Total dry weight (g)	47.5	59.8	25.51
10	Grain type	Medium Bold	Medium Bold	Medium slender

4. Black Gora (IC640862; INGR23004), a rice germplasm with tolerance to submergence with high anaerobic germination potential

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Black gora (IC640862) rice (*Oryza sativa* L.) is an early maturing upland *aus* landrace collected from Jharkhand (Roy *et al.,* 2021a). It has been characterized to possess a high level of tolerance to major abiotic stresses like drought, submergence (at both germination and seedling stages), and low-phosphorus conditions in multilocation/ season experiments. In addition, it is also resistant to blast disease. IC640862 showed a tolerant reaction (SES = 3-5) to vegetative-stage drought stress (Roy *et al.,* 2021b). Under osmotic stress induced by 1% and 2% Mannitol, it showed superior tolerance response in terms of lesser reduction (20-25%) in root and shoot biomass and lengths. It maintains a higher chlorophyll content under osmotic stress. Screening with DTY (grain yield under drought) QTL-linked markers indicated the possible presence of *qDTY2.2* in this accession. IC640862 showed, on average, 65% survival rate in multilocation trials. It possesses the *SUB1A-1* gene and follows a 'quiescence' strategy for survival under submergence

similar to FR13A. Under germination stage flooding, these accessions showed high anaerobic germination (AG) potential of 75% and maintained superior seedling vigour. IC640862 is positive for *PSTOL1* and carries Kasalath-type gene haplotype at the *Pup1* locus responsible for higher phosphorus uptake efficiency under low-P conditions. Hydroponic experiment showed that this Black gora accession produces higher root and shoot biomass and longer roots under P-limited conditions. Furthermore, IC640862 is characterised as resistant to blast disease at Hazaribag location and is found positive for the *Pi- 54* locus based on gene-specific InDel marker. Agro-morphologically, IC640862 has high vegetative vigour, intermediate plant height (121 cm), early maturing (90-95 days) and short bold kernels

References

Roy S, A Banerjee, J Kumar, BC Verma and NP Mandal (2021a) Morpho-agronomic and Genetic Diversity of Gora Rice (*Oryza sativa* L.) Landraces of Chotanagpur Plateau Region in Eastern India. *Indian J. Plant Genet. Resour.* 34(2): 196-205.

Roy S, BC Verma, A Banerjee, J Kumar, US Ray and NP Mandal (2021b) Genetic diversity for drought and low phosphorus tolerance in rice (*Oryza sativa* L.) varieties and donors adapted to rainfed drought prone ecologies. *Sci. Rep.* 11: 13671.

5. RPbio4918-166S (IC648977; INGR23005), a rice germplasm with high photosynthetic rate and high seedling vigour

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Leaf photosynthetic traits (Fig. 4) were evaluated in a set of high-yielding *Oryza sativa* cv. Swarna / *Oryza nivara* backcross introgression lines (BILs) along with recurrent parent Swarna, both in wet (*Kharif*) and dry (*Rabi)* seasons in normal irrigated field conditions. BILs 166s with high and consistent photosynthetic rate exhibited stable high yield levels and biomass production in both the seasons compared to the recurrent parent Swarna (Rao *et al.,* 2018). 166S showed high PN (23.58 lmol (CO2) m-2 s -1]) and gs 0.501 [mmol (H2O) m-2 s -1] in *Kharif* and transpiration rate (E) [mmol (H2O) m-2 s -1.

Fifteen BILs were evaluated for three seasons (*kharif* 2014, *rabi* 2015 and *kharif* 2015) for seedling vigour and yield-related traits. 166S showed higher germination percentage, Root length, seedling vigour index, and harvest index than 248S (DRRDhan40) and Swarna. Of all these 15 BILs, 166S and derived lines were already identified as stable genotypes in the previous study and showed good seedling vigour. High-yielding BIL 166S showed higher seedling vigour indices compared with checks Tulasi and Sahbhagidhan. 166S showed a QTL region linked to seedling vigour from *O. nivara* at the chromosomal region between RM223 and RM210. In terms of vigour, 166S and derived lines were better compared to checks. Introgressions of *O. nivara* into Swarna revealed transgressive segregants for all the traits studied. 166S (G6) were identified to be stable and ideal genotypes for bulk yield, grain number and number of filled grains. 166S (G6) proved significantly superior in yield over the recurrent parent Swarna and on par with the best check MTU1010. G6 (166S) showed yield stability across the seasons along with high mean yield performance. G6 is a late duration genotype, with strong culm strength, high grain number and panicle weight. G6 has a higher yield and stability than Swarna but has the Swarna grain type. 166S exhibited superior performance under direct seeded condition and conservation agriculture practices. BIL 166S, designated as IET21938 (RPBio4918-166S), was tested in 12 saline, alkaline locations in India and it was better than best check CSR36 for alkaline areas at one location, Kumarganj (UP). 166s was reported as salinity-tolerant based on their seed germination and seedling growth response under different salinity treatments. The attenuation in yield potential was comparatively low in 166S due to its efficient compartmentalization of Na+ in leaf tissue (Ganeshan *et al.,* 2016).

References

Ganeshan P, J Ajay, B Parmar, AR Rao, K Sreenu and P Mishra (2016) Identification of salt tolerant rice lines among interspecific BILs developed by crossing *Oryza sativa/O. rufipogon* and *O. sativa/O. nivara*. *Aust. J. Crop Sci.* 10:

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Rao YV, D Balakrishnan, KR Addanki, S Mesapogu, TV Kiran, D Subrahmanyam, S Neelamraju and SR Voleti (2018) Characterization of backcross introgression lines derived from *Oryza nivara* accessions for photosynthesis and yield. *Physiol Mol Biol Plants.* 24: 1147- 1164.

6. IR 129477-902-121-10-1-1 (IC648593; INGR23006), a rice germplasm with multiple biotic stress resistance genes

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The traditional system of rice (*Oryza sativa* L.) cultivation is largely dependent upon labour, water, and energy. The higher cost of pumps, depletion of groundwater, increasing labour wages, and changing climatic conditions are the most important constraints to the puddled system of rice cultivation. Direct seeding of rice is becoming the most popular method for the production of rice, as it requires less water, labour, and energy for the production of rice compared to the traditional method. To combine anaerobic germination, blast resistance, brown plant hopper resistance, bacterial blight resistance, gall midge resistance, grain yield under direct-seeded cultivation conditions, early vigour, nodal roots, and early and uniform emergence in the background of high-yielding irrigated rice cultivar IR09N538 (IRRI 132/PR 30138-35-2//IR04N114) with preferable grain type, a complex crossing program began in the 2014DS at IRRI with 12 donors, including donors for biotic and abiotic stress tolerance and direct seeded aerobic-adapted traits (Sandhu *et al.,* 2021). A total of 38 promising breeding lines were tested at PAU Ludhiana (3 years), ISARC Varanasi (3 years) and BAU Sabour (2 years). The breeding line IR 129477-902-121-10-1-1 showed grain-yield and had the following genes as assessed by molecular markers: **Xa4*: gene for BLB resistance, *BPH3*: gene for resistance to brown plant hopper, *GM4*: gene for resistance to gall midge, *Pita:* gene for blast resistance*, qAG9.1*: QTL for anaerobic germination, *qDTY3.1*: QTL for increased yield under drought conditions, *qGY6.1, qGY10.1*: QTL for increased yield under direct seeded cultivation condition, *qNR5.1, qNR4.1:* QTL associated with nodal root number facilitating nutrient uptake under variable anaerobic aerobic soil conditions. This germplasm can serve as a valuable donor for future breeding programmes

Reference

Sandhu N, S Yadav, M Catolos, MT Cruz and A Kumar (2021) Developing climate-resilient, direct-Seeded, adapted multiple-stress-tolerant rice applying genomics-assisted breeding. *Front. Plant Sci.* 12: 1-17.

7. IR 129477-709-375-3-5-7 (IC648594; INGR23007), a rice germplasm with multiple biotic stress resistance genes

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The traditional system of rice (*Oryza sativa* L.) cultivation is largely dependent upon labour, water, and energy. The higher cost of pumps, depletion of groundwater, increasing labour wages, and changing climatic conditions are the most important constraints to the puddled system of rice cultivation. Direct seeding of rice is becoming the most popular method for the production of rice as it requires less water labour, and

energy for the production of rice compared to the traditional method. To combine anaerobic germination, blast resistance, brown plant hopper resistance, bacterial blight resistance, gall midge resistance, grain yield under direct-seeded cultivation conditions, early vigour, nodal roots, and early and uniform emergence in the background of high-yielding irrigated rice cultivar IR09N538 (IRRI 132/PR 30138-35-2//IR04N114) with preferable grain type, a complex crossing program began in the 2014DS at IRRI with 12 donors, including donors for biotic and abiotic stress tolerance and direct seeded aerobic-adapted traits (Sandhu *et al.*, 2021). A total of 38 promising breeding lines were tested at PAU Ludhiana (3 years), ISARC Varanasi (3 years) and BAU Sabour (2 years). The breeding line IR 129477-709-375-3-5-7 showed grain-yield and had the following genes as assessed by molecular markers: **GM4*: gene for resistance to gall midge, *Pita:* gene for blast resistance, *qAG9.1*: QTL for anaerobic germination, *qDTY3.1*, *qDTY12.1*: QTL for increased yield under drought conditions, *qGY6.1:* QTL for increased yield under direct seeded aerobic cultivation conditions *qNR5.1:* QTL associated with nodal root number facilitating nutrient uptake under variable anaerobic aerobic soil conditions. Thus, this germplasm can serve as a valuable donor for future breeding programmes.

Reference

Sandhu N, S Yadav, M Catolos, MT Cruz and A Kumar (2021) Developing climate-resilient, direct-Seeded, adapted multiple-stress-tolerant rice applying genomics-assisted breeding. *Front. Plant Sci.* 12: 1-17.

8. IR 129477-1629-14-1-4-2 (IC648595; INGR23008), a rice germplasm with biotic resistance genes

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Direct-seeded rice (DSR) is a sustainable alternative to puddled transplanted rice (PTR) due to its better adaptability to the future labour-water shortage scenario. The DSR method of rice (*Oryza sativa* L.) cultivation is better suited to bring mechanization opportunities in agriculture and attract youths to select farming as a career. To combine anaerobic germination, blast resistance, brown plant hopper resistance, bacterial blight resistance, gall midge resistance, grain yield under direct-seeded cultivation conditions, early vigour, nodal roots, and early and uniform emergence in the background of high-yielding irrigated rice cultivar IR09N538 (IRRI 132/PR 30138-35-2//IR04N114) with preferable grain type, a complex crossing program began in the 2014DS at IRRI with 12 donors, including donors for biotic and abiotic stress tolerance and direct seeded aerobic-adapted traits. At each generation, phenotypic plant selection was conducted initially based on plant type, duration, plant height, number of tillers, grain type, and visual yield, and then selected plants were evaluated genotypically with the trait-associated markers (Sandhu *et al.,* 2021). A total of 38 promising breeding lines were selected and evaluated in multi-environments. The breeding line IR 129477-1629-14-1-4-2 showed better grain-yield with preferable grain type (Length/Breadth ratio: 3.62) and quality traits (milled rice recovery: 68.82%, head rice recovery: 59.76%) under direct seeded aerobic cultivation conditions across years. The breeding line IR 129477-709-375-3-5-7 showed grain-yield and had the following genes as assessed by molecular markers: **Xa4, xa5, Xa21*: genes for BLB resistance, *BPH3*: gene for resistance to brown plant hopper, Pi9, *Pita:* gene for resistance to blast, *qAG9.1*: QTL for anaerobic germination, *qDTY3.1*: QTL for increased yield under drought conditions, *qNR5.1, qRHD1.1:* QTL associated with nodal root number and root hair density facilitating nutrient uptake under variable anaerobic aerobic soil conditions, *qEMM1.1*: QTL for early and uniform emergence early uniform emergence

Reference

Sandhu N, S Yadav, M Catolos, MT Cruz and A Kumar (2021) Developing climate-resilient, direct-Seeded, adapted multiple-stress-tolerant rice applying genomics-assisted breeding. *Front. Plant Sci.* 12: 1-17.

9. IR 129477-1629-210-4-4-4 (IC648596; INGR23009), a rice germplasm with multiple biotic resistance genes

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Direct-seeded rice (DSR) is a sustainable alternative to puddled transplanted rice (PTR) due to its better adaptability to the future labour-water shortage scenario. The DSR method of rice (*Oryza sativa* L.) cultivation is better suited to bring mechanization opportunities in agriculture and attract youths to select farming as a career. To combine anaerobic germination, blast resistance, brown plant hopper resistance, bacterial blight resistance, gall midge resistance, grain yield under direct-seeded cultivation conditions, early vigour, nodal roots, and early and uniform emergence in the background of high-yielding irrigated rice cultivar IR09N538 (IRRI 132/PR 30138-35-2//IR04N114) with preferable grain type, a complex crossing program began in the 2014DS at IRRI with 12 donors, including donors for biotic and abiotic stress tolerance and direct seeded aerobic-adapted traits. At each generation, phenotypic plant selection was conducted initially based on plant type, duration, plant height, number of tillers, grain type, and visual yield, and then selected plants were evaluated genotypically with the trait-associated markers (Sandhu *et al*., 2021). A total of 38 promising breeding lines were selected and evaluated in multi-environments. The breeding line IR 129477-1629-210-4-4-4 showed better grain yield with a preferable grain type (Length/Breadth ratio: 3.67) and quality traits (milled rice recovery: 68.17%, head rice recovery: 54%) under direct-seeded aerobic cultivation conditions across years. The developed advanced breeding line possesses the QTL/gene for biotic and abiotic stress resistance/tolerance, a genomic region conferring early and uniform seedling establishment and root traits improving nutrient uptake under DSR. It also tested positive for markers of the following genes: **xa5, Xa21*: genes for BLB resistance, *BPH3*: gene for resistance to brown plant hopper, *Pita:* gene for resistance to blast *qAG9.1*: QTL for anaerobic germination, *qDTY2.1, qDTY3.1*: QTL for increased yield under drought conditions, *qNR5.1, qRHD1.1:* QTL associated with nodal root number and root hair density facilitating nutrient uptake under variable anaerobic aerobic soil conditions*, qEMM1.1*: QTL for early and uniform emergence early uniform emergence.

Reference

Sandhu N, S Yadav, M Catolos, MT Cruz and A Kumar (2021) Developing climate-resilient, direct-Seeded, adapted multiple-stress-tolerant rice applying genomics-assisted breeding. *Front. Plant Sci.* 12: 1-17.

10. IR 129477-3343-500-36-5-1 (IC648597; INGR23010), a rice germplasm with biotic resistance genes *Xa4+xa5+xa13 +GM4+Pita.* QTL markers (*AG9.1, qDTY3.1, qRHD1.1 and qEMM11.1*)

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In view of the urgency to save groundwater resources on one hand and food security as well as farmers' livelihood on the other, technologies aimed at saving water in rice (*Oryza sativa* L.) cultivation hold significance. Direct Seeded Rice (DSR) is a promising technology with water and labour-saving possibilities. (Sandhu *et al*., 2021). To combine anaerobic germination, blast resistance, brown plant hopper

resistance, bacterial blight resistance, gall midge resistance, grain yield under direct-seeded cultivation conditions, early vigour, nodal roots, and early and uniform emergence in the background of high-yielding irrigated rice cultivar IR09N538 (IRRI 132/PR 30138-35-2//IR04N114) with preferable grain type, a complex crossing program began in the 2014DS at IRRI with 12 donors, including donors for biotic and abiotic stress tolerance and direct seeded aerobic-adapted traits. At each generation, phenotypic plant selection was conducted initially based on plant type, duration, plant height, number of tillers, grain type, and visual yield, and then selected plants were evaluated genotypically with the trait-associated markers (Sandhu *et al.,* 2021). A total of 38 promising breeding lines were selected and evaluated in multi-environments. The breeding line IR 129477-3343-500-36-5-1 showed better grain-yield and preferable grain type (Length/Breadth ratio: 3.50) and quality traits (milled rice recovery: 64.16%, head rice recovery: 77.44%) under direct seeded aerobic cultivation conditions across years. It also tested positive for markers of the following genes: *Xa4, xa5, xa13*: genes for BLB resistance, *GM4*: gene for resistance to gall midge, *Pita:* gene for blast resistance*, qAG9.1*: QTL for anaerobic germination, *qDTY3.1*: QTL for increased yield under drought conditions, *qRHD1.1:* QTL associated with root hair density facilitating nutrient uptake under variable anaerobic aerobic soil conditions, *qEMM11.1*: QTL for early and uniform emergence.

Reference

Sandhu N, S Yadav, M Catolos, MT Cruz and A Kumar (2021) Developing climate-resilient, direct-Seeded, adapted multiple-stress-tolerant rice applying genomics-assisted breeding. *Front. Plant Sci.*12: 1-17.

11. IR 129477-4026-249-15-1-2 (IC648598; INGR23011), a rice germplasm with multiple biotic resistance genes

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The transition of the traditional puddled system of rice (*Oryza sativa* L.) cultivation to direct seeded cultivation system will be greatly facilitated by the development and adoption of new direct seeded aerobic adapted rice varieties. At PAU, a complex crossing program began in the 2014DS at IRRI with 12 donors, including donors for biotic and abiotic stress tolerance and direct seeded aerobic-adapted traits. At each generation, phenotypic plant selection was conducted initially based on plant type, duration, plant height, number of tillers, grain type, and visual yield, and then selected plants were evaluated genotypically with the trait associated markers (Sandhu *et al.,* 2021). A total of 38 promising breeding lines were selected and evaluated in multi-environments. The breeding line IR 129477-4026-249-15-1-2 carrying multiple QTL/genes viz. *Xa4, Xa21*: gene for BLB resistance, *BPH3*: resistance to brown plant hopper, *GM4*: resistance to gall midge, *qAG9.1*: QTL for anaerobic germination, *qDTY3.1*, *qDTY12.1*: QTL for increased yield under drought conditions, *qRHD1.1, qRHD5.1:* QTL associated with root hair density facilitating nutrient uptake under variable anaerobic aerobic soil conditions, *qEMM11.1*: QTL for early and uniform emergence early uniform emergence, performed well in terms of grain yield and adaptability under DSR and also exhibited phenotypically high levels of tolerance/ resistance to abiotic and biotic stresses with desired grain quality characteristics.

Reference

Sandhu N, S Yadav, M Catolos, MT Cruz and A Kumar (2021) Developing climate-resilient, direct-Seeded, adapted multiple-stress-tolerant rice applying genomics-assisted breeding. *Front. Plant Sci.*12: 1-17.

12. IR 129477-4139-439-1-1-2 (IC648599; INGR23012), a rice germplasm with Biotic resistance genes *Xa4, xa5, Xa21, Pi9, Pita*. QTL markers (*AG9.1, qDTY3.1, qDTY12.1 and qEMM11.1*)

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The transition of traditional puddled system of rice (*Oryza sativa* L.) cultivation to direct seeded cultivation system will be greatly facilitated by the development and adoption of new direct seeded aerobic adapted rice varieties. At PAU, a complex crossing program began in the 2014DS at IRRI with 12 donors, including donors for biotic and abiotic stress tolerance and direct seeded aerobic-adapted traits. At each generation, phenotypic plant selection was conducted initially based on plant type, duration, plant height, number of tillers, grain type, and visual yield, and then selected plants were evaluated genotypically with the trait associated markers (Sandhu *et al.,* 2021). A total of 38 promising breeding lines were selected and evaluated in multi-environments. The breeding line IR 129477-4139-439-1-1-2 carrying multiple QTL/genes, viz. Xa4, xa5, Xa21: genes for BLB resistance, Pi9, Pita: genes for resistance to blast, qAG9.1: QTL for anaerobic germination, qDTY3.1, qDTY12.1: QTL for increased yield under drought conditions, qEMM11.1: QTL for early and uniform emergence, performed well in terms of grain yield and adaptability under DSR and also exhibited phenotypically high levels of tolerance/resistance to abiotic and biotic stresses with desired grain quality characteristics.

Reference

Sandhu N, S Yadav, M Catolos, MT Cruz and A Kumar (2021) Developing climate-resilient, direct-Seeded, adapted multiple-stress-tolerant rice applying genomics-assisted breeding. *Front. Plant Sci.* 12: 1-17.

13. IR 129477-3343-500-36-5-1 (IC648597; INGR23010), a rice germplasm with biotic resistance genes Xa4+xa5+xa13 +GM4+Pita. QTL markers (AG9.1, qDTY3.1, qRHD1.1 and qEMM11.1)

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The transition of the traditional puddled system of rice (*Oryza sativa* L.) cultivation to direct seeded cultivation system will be greatly facilitated by the development and adoption of new direct-seeded aerobic-adapted rice varieties. At PAU, a complex crossing program began in the 2014DS at IRRI with 12 donors, including donors for biotic and abiotic stress tolerance and direct-seeded aerobic-adapted traits. At each generation, phenotypic plant selection was conducted initially based on plant type, duration, plant height, number of tillers, grain type, and visual yield, and then selected plants were evaluated genotypically with the trait-associated markers (Sandhu *et al.,* 2021). A total of 38 promising breeding lines were selected and evaluated in multi-environments. The breeding line IR 129477-4197-209-2-2-2 introgressed with multiple genes, *viz.* 8 QTL/genes* (*Xa4+xa5+ Xa21+Pita+Pita2+qAG9.1+ qDTY3.1+qNR5.1*) showed grain-yield of 5.9 t ha-1 in Philippines (4.4 t ha- 1 IR09N538) and 4.5 t ha-1 in Ludhiana location of Punjab (4.2 t ha-1 PR126) and 5.1 t ha-1 at ISRAC, Varanasi (UP) (4.4 t ha-1 IR09N538, 4.0 t ha-1 Sahbhagi dhan, 4.2 t ha-1 MTU1010) with preferable grain type (Length/Breadth ratio: 3.57) and quality traits (milled rice recovery: 71.58%, head rice

recovery: 60.28%) under direct seeded aerobic cultivation conditions across years. The genomics-assisted breeding line with a combination of root traits improving nutrient uptake, anaerobic germination and biotic/abiotic stress tolerance/resistance QTL/genes showed better yield advantages, viz. 34% in the Philippines, 5% in Punjab and 16% at ISARC Varanasi over the best performing check/recipient variety with better grain quality under direct seeded cultivation condition *viz., Xa4, xa5, Xa21*: genes for BLB resistance, *Pita, Pita2:* genes for blast resistance*, qAG9.1*: QTL for anaerobic germination, *qDTY3.1*: QTL for increased yield under drought conditions, *qNR5.1:* QTL associated with nodal root number facilitating nutrient uptake under variable anaerobic aerobic soil conditions.

Reference

Sandhu N, S Yadav, M Catolos, MT Cruz and A Kumar (2021) Developing climate-resilient, direct-Seeded, adapted multiple-stress-tolerant rice applying genomics-assisted breeding. *Front. Plant Sci.* 12: 1-17.

14. MTU 1184 (IC648601; INGR23014), a rice germplasm with Submergence tolerance

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Coastal rice (*Oryza sativa* var. indica) ecosystems, covering more than 16 per cent of rice area worldwide (20 million ha), are adversely affected by annual flooding (Panda and Barik *et al.,* 2021). Paddy fields in these flood-prone lowlands are subjected to either flash floods (a few days to two weeks) or stagnant flooding with water levels of 30-50 cm in the fields (Sem-Deep Water). In recent years, climate change has contributed to the increased incidence of both types of floods and the yield loss under such situations ranges from 10 to 100%, depending on the cultivated variety, stage of the crop, flood duration, and depth of the floodwater (Sukantha *et al.,* 2020). Keeping this in view, Regional Agricultural Research Station (RARS), Maruteru of Acharya NG Ranga Agricultural University (ANGRAU) has developed flood-tolerant variety MTU 1184 by conventional Plant Breeding using PLA 1100, released and widely cultivated as flood and submergence tolerant variety and BM 71, BPH resistant genotype, as parents from 2008 to 2011. The flood tolerant rice culture was intensively evaluated for yield at the research station and different locations under AICRIP system, in semi-deep-water ecosystem.

The entry MTU 1184 (IET 24486) was tested in station yield trials from *kharif* 2012 to *kharif* 2014 under a semi-deep-water ecosystem at RARS, Maruteru. It had 150 days duration, is tolerant to flash floods for 15 days at tillering stage and is also suitable for stagnant flooding (30-50 cm). It has medium slender grain with brown glume. The plant is semi-tall with 130 cm height and has moderate elongation ability, kneeing ability, two weeks of seed dormancy and good grain quality characteristics.

MTU 1184 is also moderately tolerant to Brown Plant Hopper (Score 5), Blast (Score 5), possessing blast resistant genes *piks, pikh, pi1, pi39, pi2* and Bacterial Leaf Blight (Score 5) as per the data from National Screening Nurseries of AICRIP.

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15. MTU IJ 206-7-4-1; (BM 71) (IC648602; INGR23015), a rice germplasm with resistance to Brown Plant Hopper

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The line, MTU IJ 206-7-4-1 (BM 71) is of medium duration (135 days), average height (140cm), profuse tillering, good grain number (205/panicle), average test weight (21.5g), long slender (3.2 L/B ratio) with light brown husk, translucent kernel and resistant to Brown Plant Hopper. The resistance was confirmed in the screening studies at different locations in the All India Co-ordinated Rice Improvement Program from 2003 to 2005. The resistance was stable across locations and years during the different periods of testing. Antixenosis (Non-preference) and antibiosis resistance mechanisms were found to be responsible for BPH resistance in BM 71. Molecular studies revealed the presence of *Bph3* and *Bph6* BPH resistance genes in MTU IJ 206-7-4-1(BM 71).

MTU IJ 206-7-4-1 (BM 71) is resistant to Brown Plant Hopper with a damage score of 3.0. Further, the resistance was confirmed in the screening studies at different locations in the All India Co-ordinated Rice Improvement Program from 2003 to 2005 (DRR Annual Progress Report 2003, 2004 and 2005). Vijaya Lakshmi *et al.,* (2010) in their studies on phenotypic response of 16 rice genotypes to brown plant hoppers (BPH) at flowering stage under field conditions reported damage score of 1 for BM 71 on 0-9 scale of SES for rice (IRRI, 2014) on par with the resistant check, PTB 33. (Vasanthabhanu *et al.,* 2014) reported antixenosis (Non-preference) and antibiosis as the resistance mechanisms operating in BM 71 for BPH resistance. (Sri Chandana *et al.,* 2015) in their molecular studies revealed the presence of *Bph3* and *Bph6* BPH resistance genes.

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16. CSAR 7-9-2020 (IET 29356) (IC648979; INGR23016), a rice germplasm with tolerance against soil sodicity

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In order to develop superior and stress tolerant rice (*Oryza sativa L.*) genotypes under medium to high alkalinity stress with a pH range of 8.5 to 10.9, rigorous screening and selections were made during Kharif 2019 in the breeding materials supplied by ICAR-IIRR, Hyderabad. After evaluation of the selected lines in the station trial, two promising genotypes, i.e. CSAR 7-9-2020 and CSAR 12-10-2020, were nominated for testing in coordinated varietal trials in Kharif 2020. Advanced Varietal Trial-1 (AL & ISTVT) comprising fourteen entries excluding six checks was conducted at 18 locations during Kharif 2021but data from six locations, namely Kurukshetra, Anjanteli, Kanpur, Lucknow, Annamalainagar and Karaikal, were found suitable for statistical analysis (Table 1). Promising genotypes were identified based on a significant critical difference of the test entries over the checks-either best check or the early check. On an overall basis, the mean grain yield ranged from 1735 kg/ha (Pusa 44) to 3337 kg/ha (IET 29356), and days to 50% flowering varied from 79 days (IET 28606) - 99 days (Pusa 44). Plant height of the entries ranged from 81.8 cm (CSR 10) to 102.2 cm (Local

Table 1: General information about soil stress parameters and yield performance of entries under alkalinity and inland salinity in AVT 1-AL & ISTVT, *Kharif,* 2021

Location/state Alkalinity	Soil pH	Soil EC (dSm-1)	Exptl. mean (Kg/ha)	Grain Yield Range (Kg/ha)	CV%	Remarks
Kurukshetra (Haryana)	9.7	1.0	3335	1527-4107	13.3	Data considered for analysis
Anjanteli (Haryana)	9.7	0.9	3375	1400-4240	10.29	Data considered for analysis
Annamalainagar (Tamil Nadu) (Tamil Nadu)	8.5	1.5	2896	1616-3350	10.1	Data considered for analysis
Lucknow (Uttar Pradesh)	9.6	0.96	2491	1014-3722	23.85	Data considered for analysis
Kanpur (Uttar Pradesh)	9.8	0.7	2235	1318-2929	19.2	Data considered for analysis
Karaikal (Puducherry)	8.5	1.8	2822	1833-3618	12.9	Data considered for analysis

Source: Annual Progress Report 2021 Vol. 1- Varietal Improvement, page no.– 1.517 (ICAR-IIRR, Hyderabad). Soft copy of the report is available on the IIRR website.

check), and the panicles/m2 varied from 288 (Pusa 44) to 349 (IET 29353). Among the checks, local check (2908 kg/ha) recorded the highest yield followed by CSR 10 (2668 kg/ha), FL 478 (2529 kg/ha), CSR 36 (2518 kg/ha), CSR 23(2424 kg/ha) and Pusa 44 (1735 kg/ha).

Among the test entries, IET 29356 (CSAR-7-9-2020), a selection from BC-3-7-9 (CST7-1/IRGC- 69861//Pusa-1601), recorded a mean grain yield of 3337 kg/ha, 91 days to 50% flowering, 97 cm plant height and 329 panicles/m^2 and ranked first on an overall basis with 15% yield superiority over the best check. This culture is also found to be superior in zone II encompassing Uttar Pradesh and Haryana states, with a mean grain yield of 3604 kg/ha and 84 days to 50% flowering. Quality-wise, IET 29356 recorded long bold grains with 68.1% head rice recovery, low amylose content of 19.36%, medium GC (47 mm) and high ASV (7).

17. DBW400 (IC648603; INGR23017), a wheat germplasm with resistant to leaf rust

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Wheat (*Triticum aestivum* L.) is prone to a number of rust diseases, namely leaf rust, stem rust and stripe rust. This rust can cause yield losses up to 80% under favourable environmental conditions. There is a need to identify the genotypes with built tolerance against rusts. These resistant genotypes can be used in the breeding programme as a donor for the development of rust resistant, high yielding wheat varieties. Leaf rust is one of the important diseases of wheat, prevailing in all the wheat growing zones of the country.

DBW400(KUTZ//KFA/2*KACHU) was selected from the CIMMYT nursery 12th STEMRRSN6023. This genotype was found highly resistant to leaf rust during different years of multi-location testing in coordinated trials (EIGN, IPPSN and PPSN) conducted under artificial epiphytotic conditions. This genotype was tested in 12 hotspot locations (Table 1) for leaf rust in IPPSN and PPSN. The proposed genotype was found to be highly resistant to leaf rust across locations and years.

Table 1: Leaf rust in IPPSN and PPSN

Year	Number of locations	Leaf rust (South)		Leaf rust (North South)	
		HS	ACI	HS	ACI
2020(IPPSN)	12	5S	1	5S	1.5
2021(PPSN)	12	tR	0.1	10S	2.9

HS: Highest score; ACI: Average coefficient of infection

Additionally, the genotype was also resistant to stem rust with HS:20S; ACI:6.1 in 2021 and HS:10S andACI:305 in IPPSN and PPSN during 2020 and 2021. Therefore, DBW400 will be a potential source to be used as a donor to incorporate genes for leaf rust resistance in an improved wheat variety.

18. UASQ 332 (IC648604; INGR23018), a wheat germplasm with high zinc content

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Worldwide, over 2 billion people suffer from iron (Fe), zinc (Zn) and/or other (multiple) micronutrient deficiencies. In India, 48% of children aged 5-10 years have zinc/iron or other micronutrient deficiencies. Zinc is one of the important micronutrients for normal growth and development. Dicoccum wheat is known to accumulate more Zn and Fe than bread and durum wheat. It also has a higher content of Protein, lysine, and crude fibre. As a consequence of these nutritional characteristics, Dicoccum wheat (DDK 1001) was used as donor for improvement of Durum wheat (HD4501). Wheat (Triticum aestivum L.) UASQ 332 was developed at the University of Agricultural Sciences, Dharwad by Gamma irradiation of F1 (DDK 1001/HD 4501) with 150Gy. The genotype was evaluated along with 53 entries at 13 different locations across the country for different quality traits in Quality Component and Wheat Bio-fortification Nursery (QCWBN), and the grain zinc data is presented in Table 1. UASQ 332 was found to be superior for grain zinc concentration (47.3 ppm) over all the check varieties. UASQ 332 was found to be superior to all 4 checks in all 4 zones. The per cent superiority of UASQ 332 over the check varieties WB02, DBW187, DBW222, and GW322 was 16.8%, 43.4%,32.3%, and 25.4%, respectively (Table 1).

The genotype also contains relatively higher grain iron concentration and grain protein content.

Thus, UASQ 332 would be a potential source to be utilized in future breeding programs to develop high yielding durum wheat varieties with enhanced grain zinc concentration.

Table 1: Grain Zinc content (ppm) of QCWBN entries

Zone	Location	Genotype	Check Varieties			
		UASQ 332	Wb02	DBW187	DBW222	Gw322
NWPZ	Zonal Mean	52.8	44.4	36.8	39.2	40.0
NEPZ	Kanpur	44.4	31.1	28.5	32.3	35.6
	Varanasi	36.7	27	26.8	25.7	29.8
	Sabour	33.3	27.1	23.9	25.1	28.3

	Zonal Mean	38.1	28.4	26.4	27.7	31.2
CZ	Indore	41.3	42.6	36.2	39.7	43.7
	Vijapur	55	53	33.1	34.7	43.4
	Powarkheda	39.7	35.2	28.2	36.2	35.6
	Zonal Mean	45.3	43.6	32.5	36.9	40.9
PZ	Pune	57.8	54.7	44.1	35.8	51.0
	Dharwad	59.8	42.8	36.3	49.1	32.8
	Niphad	43.4	41.6	29.6	35.2	36.1
	Zonal Mean	53.7	46.4	36.7	40.0	40.0
Mean (National)		47.9	41.0	33.4	36.2	38.2
% Superiority Over Checks		16.8	43.4	32.3	25.4	

19. IC112049 (INGR23019), a wheat germplasm with Terminal heat tolerance, high productive tiller numbers, thousand grain weight and harvest index

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A comprehensive study was conducted to identify heat tolerant wheat lines from an association mapping panel of 205 wheat accessions, evaluated under late sown conditions in India at three different locations (Delhi, Hisar and Karnal) for 3 consecutive years (2015-16, 2016-17 and 2017-18). Planting was done in three replications at each location. In the field screening, data was recorded for 16 agronomic and physiological traits such as days to heading (DH), days to anthesis (DA), days to physiological maturity (DM), chlorophyll fluorescence (CFL), cell membrane stability (CMS), grain filling duration (GFD), grain weight/spike (GW, g), grain numbers/spike (GN), grain numbers/m (GNM), productive tillers/m (PTL), plant height (PHT, cm), 1,000 grain weight (TGW, g), biomass (BM, gm−2), grain yield (GY, gm−2), grain filling rate (GFR), and harvest index (HI, %) to find out the variation among studied wheat accessions. Stability analysis and AMMI biplot were also performed to analyze the stable performance of genotypes across the environment and years. Genotyping using a high density 35 K array revealed a large number of SNPs that could be used for the GWAS analysis. In total, 69 QTLs were identified for the ten agronomic traits. The favourable alleles for each QTL region were identified by comparing the extreme phenotypic values in association mapping panel. GY is the most desirable agronomic trait; however, its genetic regulation is very complex, controlled by a number of genes whose expressions are influenced by environmental conditions. In GGE biplot analysis, HI, PTL, GFD, GN, GY, and TGW traits were performed for yield components to identify the best genotypes and stability across the 3-locations i.e., Delhi, Hisar and Karnal. For PTL, TGW and HI, IC112049 germplasm was the best performing genotype at Delhi and Karnal. The analysis revealed heat tolerant germplasm lines based on the presence of high favourable alleles for the IC112049 germplasm line. This is a potential genetic resource for heat tolerance, which can be further used in the wheat improvement programme.

20. PAU16076 (IC648605; INGR23020), a wheat germplasm with resistant to yellow rust with gene *Yr5*

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The quality of wheat (*Triticum aestivum* L.) grain-based products is determined mainly by prolamins consisting of polymeric glutenin and monomeric gliadins proteins. The balanced ratio of these monomeric and polymeric proteins provides the required amount of strength and viscosity for the dough (Wang *et al.,* 2009). Wheat varieties with 1BL/1RS chromosomal translocation are quite popular throughout the world due to their better yield and adaptation, but this translocation is also responsible for lowering bread making quality. The presence of *Sec- 1* locus on the translocated arm 1RS causes dough stickiness (`sticky dough syndrome') due to presence of ω-and γ-secalins proteins encoded by 1RS locus (Lukaszewski *et al.,* 2014). To replace this locus in a popular Indian wheat cultivar PBW550 (PBW550+*Yr5*) with corresponding locus from 1BS, one NIL with absence of secalin locus (*Sec-1*-) in cultivar Pavon background was used as donor (called Pavon 44:38). BC2F5 and BC2F6 NILs with absence of secalin locus were evaluated for two years in replicated yield trial and NILs were tested for bread making quality. NILs were identified having reduced dough stickiness with performance of NIL pau16076 (NIL 72) for bread making characteristics was superior to parental line PBW550 with better loaf volume in the range of 580cc. This line is giving yield of 21.0 qt/acre. In the process of marker assisted transfer of 1BS locus for the absence of *Sec-1*, care was taken to keep rest of 1RS chromosomal arm intact with the help of markers. The presence of rye specific chromatin in NIL 16075 was tested positive with the help of markers rye F3/R3. Besides the NIL was also tested positive for important genes of *Lr26/Yr9/Sr31* and *Pm8* of 1RS chromosomal arm. Beside the line is positive for stripe rust resistance gene *Yr5*.

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21. PAU16077 (IC648606; INGR23021), a wheat germplasm with possesses genes for resistant to leaf rust-stripe rust (*Lr57-Yr40*) and stripe rust (*Yr15*)

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Wheat (*Triticum aestivum* L.) is the most widely consumed food grain, and like other cereal crops, the yield is a complex quantitative trait determined by different parameters of tiller number, grain number, grain weight, etc. Grain or kernel weight (thousand grain weight- TGW), consisting of grain length, width, and area, has high heritability, which not only translates into higher yields but also has a favourable effect on flour yield (Gegas *et al.,* 2010)**.** Moreover, uniform and larger sized grains are visually appealing and fetch higher market prices. In the present study, pyramiding of three rust resistance genes, *viz. Yr15* and linked genes *Lr57-Yr40* along with high TGW, into wheat variety PBW550 using a "transfer first and assemble later" strategy (Ishii and Yonezawa *et al.,* 2007). High TGW has been transferred from Rye selection 111, through conventional breeding methods while the three rust resistance genes were transferred through marker assisted selection (MAS).

High TGW was transferred into PBW550 through conventional backcross breeding with high grain weight version of PBW343. High grain weight in PBW343 has been transferred from a selection called Rye SelIII. Phenotypic selections in three-year replicated trials led to an increase in TGW of 18%. Improved versions of PBW550 with increased TGW of 45.5 was crossed sequentially with PBW550+*Yr5* (PBW746), with stripe rust resistance and

PBW550+*Lr57-Yr40* (BWL5236) line, having linked stripe rust- leaf rust resistance genes. The line PAU16077 has been tested at three locations in Punjab for two years, and an average yield of 22.1 qt/acre was recorded.

Resistance to leaf rust and stripe rust has been incorporated in the high TGW version of PBW550 through marker assisted pyramiding of stripe rust resistance gene *Yr15* using marker *Xuhw302*, and a pair of linked leaf rust and stripe rust resistance genes *Lr57-Yr40* using marker *Ta5DS-2754099_kasp23* one by one.

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22. PAU16078 (IC648607; INGR23022), a wheat germplasm with resistance to leaf rust and stripe rusts

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Among the different biotic stresses of wheat (*Triticum aestivum* L.), the stripe rust and leaf rust impose major threats to yield (Singh *et al.,* 2020). As the pathogen evolves quickly, leading to the breakdown of many major genes, a strong backup of resistance genes is required to tackle the future threats quickly. *Aegilops triuncialis* (UtUtCtCt), the non-progenitor of wheat, is an excellent source of biotic and abiotic stress resistance. The leaf rust (LR) and stripe rust (YR) resistant accession of *Ae. triuncialis*, pau 3462 was crossed and backcrossed susceptible cultivar WL711(NN) by inducing homeologous pairing using CS *ph1*. BC2F7 introgression line with 2n chromosome number of 42, with resistance to LR and YR, was selected and named as IL*tri*. IL*tri* was crossed with leaf rust and stripe rust susceptible cultivar WL711(NN), and F2 and F2:3 mapping populations were developed. These populations were screened against leaf rust and stripe rust pathotypes at the seedling and adult plant stage and found to be segregated into 3R:1S (F2) and 1HR:2Seg:1HS ratios (in F3), for both leaf rust and stripe rust, indicating inheritance of a single dominant all-stage resistance gene working against both the rusts. The leaf rust and stripe rust resistance genes were temporarily designated as *Lrtri* and *Yrtri,* respectively, and found to be inherited independently of each other. Molecular mapping with 614 SSR markers from the whole genome of wheat mapped the *Lrtri* at a distance of 11.2 cM from SSR marker *Xwmc*606. These two genes, *Lrtri* and *Yrtri,* are showing resistance to all the prevalent races of leaf rust and stripe rust.

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23. PAU16075 (IC648608; INGR23023), a wheat germplasm with Glu-B3/GliB1 locus transfer on 1RS chromosomal arm, resistant to stripe rust with transfer of gene *Yr5*

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Wheat with 1BL/1RS translocation is known for providing better yield and adaptation, but is also responsible for lowering bread making quality due to reduced dough strength. This is due to absence of

wheat 1BS locus *Glu-B3/Gli-B1* encoding low molecular weight glutenins and gliadins (Wang *et al.,* 2016). In present work marker assisted selection has been utilized to introduce Glu-*B3/Gli-B1* locus in 1RS arm in wheat variety PBW550 (PBW550+*Yr*5). The donor for Glu-*B3/Gli-B1* locus was a NIL in the background of cultivar Pavon, carrying a modified 1RS arm with presence of *Glu-B3/Gli-B1* (called Pavon 40:9) (Lukaszewski *et al.,* 2014).

The phenotypic evaluation of BC2F5 and BC2F6 NILs with *Glu-B3/GliB1* positive locus was done for two years in a replicated yield trial. NILs PAU16075 was selected with an average yield of 20.6 qt/acre, having plant height of 90cm. The line is having improved dough strength with protein content of 11.76%, SDS sedimentation value of 37.72cc, dry gluten 11.50% and gluten index of 81.70. The loaf volume 565cc, dough consistency, and crumb texture of this NIL is superior to the recurrent parent PBW550 because of the retained glutenins and gliadins.

The care was taken for the retention of 1RS arm during the back-cross assisted transfer of *Glu-B3/GliB1* locus. The presence of rye specific chromatin in NIL 16075 was tested with help of markers rye F3/R3 while the important genes on 1RS arm viz. *Lr26/Yr9/Sr31* and *Pm8* were also tested positive with respective markers. The line is also resistant to stripe rust due to presence of gene *Yr5*.

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24. BFKW-2 (EC787008; INGR23024), a wheat germplasm with high grain protein (16.7%), Iron (45.7 ppm) and Zinc (47.8 ppm) content

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Zinc is one of the important micronutrients for the normal growth and development of plants and animals. A set of 14 Amphidiploids with 4 international checks received from the University of Nottingham, United Kingdom, under DBT-BBSRC project and 2 Indian checks (1 aestivum + 1 durum) were evaluated for zinc, iron, protein content, agronomic traits and disease resistance. They were planted continuously for 2 years during 2018-19 and 2019-20 in a simple randomized block design with 4 rows of 2.5 meter plots in replication at ICAR-IIWBR Hisar and ICAR- IIWBR Karnal. Pooled and environment-wise grain protein, iron, and zinc content are presented in Table 1. Agronomic traits like days to heading (DTH), days to maturity (DTM), plant height (PHT), thousand grain weight (TKW), tiller/meter, spike length (cm). Zinc and iron estimations were performed using an Oxford instrument X-Supreme 8000. BFKW-2 found to be superior with 16.7% grain protein, 45.7 ppm grain iron and 47.8 grain zinc content in comparison to check

Table 1: Pooled grain protein, iron, and zinc content in wheat genotypes

Trait	Testing genotype	Check	
	BFKW-2	DBW 187	HI 8498
Grain Protein (%)	16.7	13.7	12.8
Grain Iron (ppm)	45.7	37.3	44.8
Grain Zinc (ppm)	47.8	35.8	40.6
Per cent Superiority Over Checks			
Grain Protein	-	21.9	30.5
Grain Iron	-	22.5	2.0
Grain Zinc	-	33.5	17.7

varieties DBW 187 (Protein: 13.8%; Iron: 37.3 ppm; Zinc: 35.8 ppm) and HI 8498 (Protein: 12.8%; Iron: 44.8 ppm; Zinc: 40.6 ppm). Per cent superiority ranges from 21.9-30.5, 2.0-22.5, and - 17.7-33.5 over check varieties for grain protein, grain iron, and grain zinc, respectively. Thus, BFKW-2 would be a potential source as high grain protein, iron and zinc donor parent. This valuable germplasm may be utilized in breeding programs to develop bread wheat varieties with high protein and micronutrient content.

25. BFKW-7 (EC787015; INGR23025), a wheat germplasm with high grain protein and Zinc content

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Worldwide, over 2 billion people suffer from iron (Fe), zinc (Zn) and/or other (multiple) micronutrient deficiencies. In India, 48% of children aged 5-10 years have zinc/iron or other micronutrient deficiencies. Zinc is one of the important micronutrients for normal growth and development. A set of 14 Amphidiploids with 4 international checks received from the University of Nottingham, United Kingdom, under DBT-BBSRC project and 2 Indian checks (1 *aestivum* + 1 *durum*) were evaluated for zinc, iron, protein content, agronomic traits and disease resistance. They were planted continuously for 2 years during 2018-19 and 2019-20 in a simple randomized block design with 4 rows of 2.5 meter plots in replication at ICAR-IIWBR Hisar and ICAR-IIWBR Karnal.

Pooled and environment-wise grain protein, iron, and zinc content is presented in Table 1. Agronomic traits like days to heading (DTH), days to maturity (DTM), plant height, (PHT), thousand grain weight (TKW), tiller/meter, spike length (cm). Zinc and iron estimations were performed using an Oxford instrument X-Supreme 8000. BFKW-7 found to be superior with 17.1% grain protein, 53.3 ppm grain iron and 54.2 grain zinc content in comparison to check varieties DBW 187 (Protein: 13.8%; Iron: 37.3 ppm; Zinc: 35.8 ppm) and HI 8498 (Protein: 12.8%; Iron: 44.8 ppm; Zinc: 40.6 ppm). Per cent superiority ranges from 24.8–33.6, 18.9–42.9, and 33.5–51.4 over check varieties for grain protein, grain iron, and grain zinc, respectively.

Thus, BFKW-7 would be a potential source of high grain protein, iron and zinc donor parent. This valuable germplasm may be utilized in breeding programs to develop bread wheat varieties with high protein and micronutrient content.

Table 1: Pooled grain protein, iron and zinc content in wheat genotypes

Traits	Testing Genotype	Checks	
	BFKW-7	DBW 187	HI 8498
Grain Protein (%)	17.1	13.7	12.8
Grain Iron (ppm)	53.3	37.3	44.8
Grain Zinc (ppm)	54.2	35.8	40.6
Percent Superiority Over Checks			
Grain Protein	-	24.8	33.6
Grain Iron	-	42.9	18.9
Grain Zinc	-	51.4	33.5

26. GW A 2019-957 (IC0642305; INGR23026), a wheat germplasm with high zinc content

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All living organisms require an optimum intake of essential mineral micronutrients for their normal growth and development, and human beings obtain these essential nutrients from their daily diet (Welch and Graham *et al.,* 2004). Malnutrition due to insufficient intake of micronutrients, including Fe and Zn, has been identified as one of the major global health issues, particularly in children aged 0–5 years, and pregnant and lactating women, affecting nearly 1.5 to 2 billion people across the globe. The intensity of the risk is much higher in countries dominated by cereal-based diets, particularly in Asia and Africa (Kumssa *et al*., 2015). Genetic biofortification makes use of plant breeding techniques to produce genotypes with higher micronutrient levels, reducing levels of anti-nutrients and increasing the levels of substances that promote nutrient absorption. Accordingly, GW A 2019-957 was developed (cross DBW 31/WR 1873) and identified as a high zinc content genotype possessing high yield. The grain yield of GW A 2019-957 (64.4 q/ha) was significantly superior to the best zonal check GW 322 (50.8 q/ha) over the location in Quality Component and Wheat Biofortification Nursery (QCWBN) in the central zone, and also possessed high zinc content (47.0 ppm) as compared to the best check UP 2672 (41.7 ppm).

The mean performance of the GW A 2019-957 under QCWBN central zones revealed its superiority for a combination of traits like high zinc concentration and grain yield (Table 1), establishing its usefulness as a potential donor for biofortification in wheat.

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Table 1: Overall performance of GW A 2019-957 in QCWBN during 2020-2021

Genotype	Grain yield (q/ha)	Zinc content (ppm)
GW A 2019-957	64.4	47.0
UP 2672 (c)	51.0	41.7
MACS 6222 (c)	67.9	40.1
DBW 187 (c)	60.2	34.4
WB 2 (c)	63.1	39.0
HD 3086 (c)	49.1	36.4
GW 322 (c)	50.8	39.5
HS 490 (c)	48.6	38.3
CD at 10 %	6.9	2.4

27. HS545 (IC648609; INGR23027), a wheat germplasm with resistant to all pathotypes of Brown Rust

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Leaf rust or brown rust caused by the fungus *Puccinia triticina* Eriks has its impact on the wheat (*Triticum aestivum* L.) crop grown in 30 million hectares (mha) area of India, Pakistan, Bengladesh and Nepal (Huerta-Espino *et al.,* 2011). The pathogen is very dynamic and renders wheat cultivars susceptible by evolving new pathotypes. To counter such threats, diverse wheat germplasm carrying brown rust resistance genes effective at different stages of growth are required. In this direction, a rust resistant wheat germplasm HS545 was developed from a cross "HD2819/HS435" Bulk-Pedigree method of breeding to have parental choice with the wheat breeders. HS545 has been found resistant against all the pathotypes of brown rust, including 77-8 and 77-10, known virulence for *Lr19* and *Lr28* under seedling resistance test. Rust resistance gene complex *Lr24/Sr24,* derived from *Agropyron elongatum*, located on 3DL, confers resistance to all the currently prevalent pathotypes of brown rust in the Indian Sub-continent (Bhardwaj *et al.,* 2021). HS545 has been validated for the presence of *Lr24/Sr24* using molecular marker *Sr24#12*. Inheritance studies revealed the presence of single dominant gene pair for controlling brown rust against the virulent pathotype 77-5. HS545 possesses agronomic features *viz*., semi-erect growth habit, green foliage, waxy flag leaf sheath, waxy peduncle with bent attitude, tapering ear shape, 94cm plant height, brown ear with brown medium awns, matures in 157 days, the grains are semi-hard and ovate shaped, amber in colour with thousand grain weight of 40 g. Broad spectrum resistance present in HS545, along with good agronomic features, would serve as desirable choice for breeding brown rust resistant wheat varieties in India.

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28. DWRBG-13 (IC646834; INGR23028), a barley germplasm with higher malt beta glucanase activity (384 Units/kg malt), lower wort beta glucan content (130 ppm)

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Malt is one of the major industrial products from barley (*Hordeum vulgare* L.), which is further utilized mainly for beer production. The malt producing industry requires certain minimum quality parameters in the barley to get higher recovery and better quality. The higher beta glucans content in grain may reduce water uptake during steeping, while in wort it adversely affects filtration rate and quality of malt extract. DWRBG-13 (ICARDA-11), along with four checks, was grown at six locations during the *rabi* season of 2020-21, and grains were malted and mashed. The genotype ICARDA-11, registered the highest malt beta-glucanase activity as compared to the checks, with an average value of 384U/kg of malt (Table 1). It had a lower wort beta-glucan content of 130 ppm (Table 2). This genotype can be a potential source of these two traits in the malt improvement programme of the country.

Table 1: Activity of Beta-Glucanase (Units/kg) in malt of different barley genotypes

Genotype	Karnal	Hisar	Ludhiana	Durgapura	Pantnagar	Kanpur	Average	Average*
ICARDA-11	380	360	382	487	369	315	382	384
DWRUB 52(c)	260	279	309	308	239	280	279	286
DWRB 101 (c)	175	186	197	263	256	140	203	194
DWRB-182 (c)	167	264	257	299	240	167	232	231
DWR 37 (c)	195	223	208	269	272	272	240	235

Table 2: Wort Beta-Glucan content (ppm) in different barley genotypes

Genotype	Karnal	Hisar	Ludhiana	Durgapura	Pantnagar	Kanpur	Average	Average*
ICARDA-11	198	92	167	101	39	107	117	130
DWRUB 52 (c)	552	397	404	392	261	280	381	401
DWRB 101 (c)	471	370	602	447	232	176	383	408
DWRB-182 (c)	368	325	432	441	234	321	353	373
DWR 37 (c)	683	856	633	462	269	239	524	566

**Average values excluding Pantnagar location, which registered exceptionally low wort beta glucan content*

29. BHS 479 (IC646838; INGR23029), a barley germplasm resistant to most pathotypes of leaf rust and stripe rust at the seedling stage

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BHS 479 (BBM 798) is a barley (*Hordeum vulgare* L.) line resistant against leaf, stripe and stem rust. It has shown seedling resistance against all the prevailing pathotypes of leaf rust. The proposed genetic stock also possesses seedling resistance against all the pathotypes of stripe rust (except for 24, showing a moderately resistant response). BHS 479 (BBM 798) also showed adult plant resistance to leaf (highest score TMS), stem rust (highest score 0) and stripe rust (ACI 5.8). This line is developed following the pedigree method of breeding involving three- way crosses between barley breeding line BBM 556 with BHS 169 and a registered barley genetic stock BHS 369 at ICAR-IARI, Regional Station, CHC, Amartara Cottage, Tutikandi Centre, Shimla (H.P.).

BHS 479 (BBM 798) has a semi-erect growth habit with medium maturity (165 days) under Northern Hill conditions. The average yield is 3.46 t/ha under the rainfed condition of the Northern Hill Zone of All India Co-ordinated trials 2019-20 (Table 1). The distinguishing features of BHS 479 (BBM 798) are six-rowed, hulled; semi-erect growth habit; erect flag leaf attitude; green leaves and 1000-grain weight of 42 g.

Table 1: Reaction of BHS 369 to major diseases

Diseases	Condition	Year	Response of Proposed Genetic stock BHS 479 (BBM 798)
Leaf Rust (Resistant to	NBDSN (APR)	2019-20	HS= TMS
brown rust)	NBDSN (Seedling)	2019-20	R (Resistant to all races)
Stem Rust (Resistant to black rust)	NBDSN (APR)	2019-20	HS=0
Stripe rust (Resistant to Yellow rust)	NBDSN (APR)	2019-20	ACI=5.8
	NBDSN (Seedling)	2019-20	R to all races (except for *24* showing MR response)
	IBDSB	2018-19	Yellow rust ACI 1.0 (Highest Score 5S)

ACI= Average Coefficient of incidence HS= Highest Score
IBDSN= Initial Barley Disease Screening Nursery NBDSN= National Barley Disease Screening Nursery

30. VR 1143 (IC647589; INGR23030), a finger millet germplasm with early duration combined with neck and finger blast resistance

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Finger millet [*Eleusine coracana* (L.) Gaertn.] is drought resistant nutri cereal grown for food & fodder. The future improvement of the crop depends upon the amount of valuable trait-specific germplasm available for different traits. Finger millet, VR 1143, is early duration entry developed by crossing VR 708 with GPU 48 and followed by the pedigree method of selection. It belongs to early maturity with medium plant height and a larger number of productive tillers/plant.

The proposed entry, along with other test entries and two checks were evaluated during the four years from 2017 to 2020. VR 1143 matures six days earlier than the national early check, VL 352, while it was on par with the very early check, VR 708. The check, VR708 (63.9% & 56.2%), is highly susceptible to neck and finger blast, while the entry, VR 1143 (5.7% & 4.9%) was resistant to neck & finger blast disease, and hence this entry can be used in crop improvement for the development of early maturity varieties with resistance to blast disease.

31. VR 1135 (IC647590; INGR23031), a finger millet germplasm with banded blight resistance

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Finger millet [*Eleusine coracana* (L.) Gaertn.], VR 1135 is a Banded blight resistant line developed by crossing PS1 with VL 315 and following pedigree method of selection. The entry, VR 1135 was evaluated with other promising entries along with local (VR 847) & national checks (PR 202, VL 350) for yield and also with disease resistant (GE4999) & disease susceptible (VR 708) checks. It was tested in consecutive years from 2017 to 2020 during Kharif season in Randomized block design with three replications at the Agricultural Research Station, Vizianagaram (lat 18^{0} 10'N, long 83^{0}39' E and altitude of 74 msl), Andhra Pradesh, which is hot spot for blast & banded blight disease.

The entry, VR 1135 belongs to medium maturity (121 days) as like the local check VR 847 (124 days). The entry VR 1135 was evaluated from 2017 to 2020 for the disease severity. Scoring for the disease severity during all four years of testing was done. The entry, VR 1135 recorded significantly lower score of banded blight over all the checks during all four years of testing. It recorded 71.80%, 40.96% and 87.10% less incidence of banded blight over Local check VR 847, Resistant check (GE 4999) and Susceptible check (VR 708), respectively. It recorded fewer incidences of banded blight (3.8%) among all the 30 entries tested for banded blight resistance, and it also showed resistance to neck & finger blast (4.0% each). VR 1135 is unique in terms of banded blight resistance and also possesses resistance to neck and finger blast. Since it is an advanced breeding line, it can be used directly as a source for the development of high-yielding banded blight and blast resistant varieties.

32. VR 1125 (IC647592; INGR23032), a finger millet germplasm with neck blast resistance and finger blast resistance

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Finger millet [*Eleusine coracana* (L.) Gaertn.] is one of the small millets that is highly valued for its nutrient composition. It is a crop of both food and nutritional security. Blast is a major problem in the finger millet crop, which can hamper the production up to 70% in severe cases. Hence, finger millet improvement is oriented towards the development of blast resistant high yielding varieties. Finger millet, VR 1125 is neck and finger blast resistant, high yielding variety developed by crossing Udurumallige with GPU 48 during 2010, followed by the pedigree method of selection up to F6 generation. It was evaluated with other 23 promising lines along with local and national check for yield; resistant and susceptible check for blast disease resistance in Randomized block design with three replications during consecutive four years (*Kharif*, 2017 to 2020) at Agricultural Research Station, Vizianagaram (lat 180 10'N, long 83039' E and altitude of 74 msl), Andhra Pradesh which is hot spot for blast disease. The proposed entry, along with other test entries and two checks, was tested under high-pressure conditions under field conditions during the four consecutive four years from 2017 to 2020.

The entry VR 1125 belongs to medium maturity (115 days) with longer ear & finger length (9.1 cm & 8.5 cm) compared to the check VR 847 (7.9 cm & 7.3 cm) respectively. It recorded a significantly lower score of neck and finger blast over all the checks during all four years of testing. VR 1125 (3.5%) recorded 71.6%, 60.1% and 94.6% less incidence of neck blast over Local check (12.6%), Resistant check (8.7%) and Susceptible check (63.9%) respectively. VR 1125 (3.0%) also recorded 76.0%, 54.4% and 94.7% less incidence of finger blast over Local check (14.5%), Resistant check (6.4%) and Susceptible check (56.1%), respectively. Moreover, the entry has recorded higher fodder yield & grain yield compared to the local check, VR 847. VR 1125 is unique in terms of neck and finger blast resistance. Hence, it can be used directly as a source for the development of high yielding blast resistant variety.

33. PPR 2885 (IC595249; INGR23033), a finger millet germplasm with non-lodging having uniform maturity

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Finger millet [*Eleusine coracana* (L.) Gaertn.] line PPR 2885, a cross derivative of PPR 2709 and Kalyani, developed through the pedigree selection method, was elevated in multi-environments. It was found to be highly tolerant to blast (leaf blast, neck blast and finger blast), brown spot, leaf sheath blight and stem borer. The culture matures within 115-120 days and attains 50% flowering in 85-98 days after sowing. It has an erect plant type with semi dwarf (85-90 cm) plant height. It has light purple pigmentation at nodes. The ear head is semi compact nature and has many lengthy, top curved fingers. The colour of the grain is light purple brown with a test weight of 3.0 g. Quality of grain is good, having high calcium and Total free amino acids. At maturity, it is resistant to lodging due to culm strength semi dwarf nature of the plant.

34. WN 630 (IC648610; INGR23034), a finger millet germplasm with High number of fingers and longer ear head length

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A total of 38 finger millet [*Eleusine coracana* (L.) Gaertn.] accessions constituted of 25 promising landraces of long earhead and thirteen released varieties were evaluated for 12 morphological characters, including grain yield. Based on mean performance over three years (*Kharif* 2019, 2020 and 2021) at two locations, the genotype WN-630 showed a very high number of fingers per earhead *i.e.* 12.50, which is >8 (high) as per DUS testing guidelines and very long earhead length, *i.e.* 13.43 cm, which is >12 cm (very long) as per DUS testing guidelines. This morphological trait, such as the number of fingers per earhead, main earhead length and length of finger, has direct contributions towards the high grain yield per plant. The best promising finger millet genotypes, *viz.* WNN-630, for a high number of fingers per earhead and very long earhead length, with moderate resistance to pests and diseases found during three years of genetic evaluation at three different locations, which was used as a promising parent in a further breeding programme.

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