

DOI Link: <https://doi.org/10.61586/sod1j>
Vol.43, Issue.5, Part.1, May 2025, PP. 33-65

Deciphering the genetic diversity and combining ability of 24 distinct inbred lines of pearl millet [*Pennisetum glaucum* (L.) R. Br] for grain yield and related components.

Siva Kumar Kanchana^{1,2}, Siddhartha Maiti¹

1.School of Biosciences Engineering and Technology, VIT Bhopal University, Kothrikalan-466 114, Sehore, Madhya Pradesh, India.

2.Rallis India Limited, Hyderabad-500 070, Telangana, India

Corresponding Author: Siddhartha Maiti¹, School of Biosciences Engineering and Technology.

Email: Siddhartha.maiti@vitbhopal.ac.in

Tel: +91- 9326132839.

Received March 2025 Accepted April 2025 Published May 2025

Abstract:

Knowledge of combining ability and genetic diversity is essential for creating high-yielding hybrids suitable for commercial agriculture. This research evaluated general combining ability (GCA) and specific combining ability (SCA), selected promising hybrids, quantified genetic diversity among 24 distinct inbred lines (18 lines and 6 testers), and associated genetic distance with hybrid performance. Assessments was done on 108 F₁ hybrids with a randomised complete block design at a single location with three replications throughout the 2023 Kharif season. Both additive and non-additive gene actions contributed to the inheritance of all assessed traits; nevertheless, additive gene action was prevalent for the majority of traits. Inbred PMB 125 demonstrated significant general combining ability (GCA) effects for head weight, grain yield, single panicle weight, single panicle grain weight, and fodder yield. PMB 133 and PMB 142 also displayed positive GCA effects across multiple traits, with PMB 142 exhibiting early maturity attributed to its negative impact on days to flowering. PMB 106 considerably enhanced productive tillers and yield characteristics. PMR 247 exhibited robust performance as a general combiner for head weight per plot, grain yield, leaf breadth, single panicle grain weight,

panicle length, and fodder yield per plot among testers. The SCA analysis identified superior hybrids such as PMB115 x PMR203, PMB113 x PMR247, PMB106 x PMR230, and PMB122 x PMR203/PMB122 x PMR249, demonstrating enhanced performance in yield components and their associated attributes. High-yielding hybrids, including PMB137 x PMR248, PMB105 x PMR230, PMB123 x PMR247, and PMB142 x PMR249, are appropriate for commercial production. Genetic distance study indicated modest variation among the paternal inbred lines, with markers exhibiting varying polymorphism (average PIC 0.561). Although GD did not directly forecast hybrid performance, there were substantial associations with specific features like as plant height, single panicle grain weight, and grain yield. This study offers significant insights into the inheritance of pearl millet grain production and key features, as well as the relevance of general combining ability (GCA), specific combining ability (SCA), and genetic variation among inbred lines.

Keywords: Combining ability, genetic diversity, pearl millet, grain yield, hybrid performance

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important forage and food crop in arid and semiarid regions of India and Africa. Globally, India is the top producer of millets accounting 13.21 MT production covering 9.76 MHa, followed by China mainland (2.7MT), Niger (2.1MT), and Nigeria (1.9MT) (FAOSTAT, 2021). Pearl millet constitutes approximately two-thirds of millet production in India, accounting for 9.62 MMT in the financial year 2021-2022. (Statista, 2022). Based on climatic conditions, production constraints, and the differential requirements of various crop-growing regions, India's agricultural zones cover approximately 8.06 million hectares and include Zone A1 (3.78 m.ha), Zone A (2.85 m.ha), Zone B (1.02 m.ha), and the Summer zone (0.41 m.ha). It is widely cultivated in many Indian states, including Rajasthan, Maharashtra, Gujarat, Uttar Pradesh, Haryana, Karnataka, Madhya Pradesh, Tamil Nadu, and Andhra Pradesh. Based on data from

2012-13 to 2021-22, the average grain yield in various Indian states ranges from a minimum of 581 kg/ha in Jharkhand to a maximum of 2664 kg/ha in Gujarat (Summer), with an overall average yield of approximately 1495 kg/ha ^{*}(<https://des.agri.gov.in>). Pearl millet grain contains 5.03 to 6.0 percent fat, 1.05 to 1.7 percent crude fiber, and 65 to 70 percent carbohydrates, making it highly caloric per 100 grams due to its carbohydrate, fat, and protein content (Burton, 1972; Flech, 1981). It is rich in iron (18 to 135 ppm) and zinc (22 to 92 ppm), essential nutrients often deficient in global populations, including India Rai et al., (2012). Cultivated predominantly (>92%) in rainfed conditions, pearl millet thrives in India's arid and semi-arid regions, receiving most of its annual rainfall (150 to 750 mm) from June to September

Pearl millet, primarily grown in rain fed areas, crop faces significant challenges due to abiotic stresses, particularly drought caused by inconsistent rainfall. Developing cultivars that can suitable in these unpredictable conditions is crucial for sustainable yield production. Identifying superior parents and hybrids is key to developing new hybrids that perform well across diverse agro climates. Combining ability analysis is crucial for assessing the genetic merit of inbreds, facilitating the identification of parents that can effectively transmit favorable alleles to their progeny. The Line × Tester mating design, as introduced by Sprague and Tatum, (1942). Efficiently assesses large germplasm collections, providing valuable insights into genetic variance and effects. This study focuses on the genetics and heterosis of yield and its related traits, aim to select hybrids with enhanced resilience and productivity for varied climatic conditions

The successful identification of desirable hybrid combinations depends on the combining ability of the parents and the gene effects involved in the expression of target trait Karvar et al., (2017), Mageto, E.K et.al., (2017) Furthermore, knowledge of gene action is important to devise an appropriate breeding strategy Kamara, M.M et.al.(2014). General combining ability (GCA) and specific combining ability (SCA) are widely used in selection of good parents and hybrids, respectively Carena, M.J.al (2010). Among different biometrical approaches, the line X tester

mating design is commonly used by pearl millet breeders to estimate GCA and SCA effects Gaoh et.al (2024), Zhang, Y .et.al (2016) GCA is associated with additive gene effects, whereas SCA is typically associated with non-additive gene effects. Sprague et.al. (1942). both additive and non-additive gene actions were reported to be important in the inheritance of pearl millet grain Al-Naggar et.al. (2014).

The assessment of the diversity and genetic distance in the available pearl millet inbreds is important for a hybrid breeding program, in order to identify inbreds that would produce crosses with good levels of heterosis without testing all hybrids combinations Badu-Apraku, B (2013), Akinwale, R(2014) . Different types of DNA markers are available to estimate genetic distance. Singh et al. (2018), Ramya et al. (2018) The simple sequence repeat (SSR) markers or microsatellites have been considered as the markers of choice owing to their co-dominant, high polymorphic, multi-allelic nature and high reproducibility Sserumaga, J.P(2014).

However, contradictory results have been reported with respect to the relationship between genetic distance and hybrid performance in pearl millet. Significant correlations were reported between molecular marker-based GD and F1 hybrid grain yield in maize . Whereas, other studies reported no significant correlation Dhliwayo, T (2009), Menkir, A (2004) . Marker-based GD and hybrid yield/SCA for grain yield had no correlation, as was also reported in other studies in different crops Riday et al. (2003); Geleta et al. (2004); Teklewold and Becker (2006); Zeid et al. (2003); Gupta et al. (2018)The objectives of this study were (i) to estimate GCA of the inbred lines and SCA of the hybrids (ii) to determine the mode of gene action controlling grain yield and other important agronomic traits; (iii) to identify promising hybrids that yield well and (iv) to assess genetic diversity among twenty-four inbred lines and correlate genetic distance to hybrid performance and SCA.

The genetic diversity between crossing parents is crucial for generating variation for selection or maximising hybrid vigour. Therefore, the establishment of heterotic groupings within breeding populations is a crucial breeding objective to improve hybrid vigour. Nevertheless, there is a paucity of studies regarding the evaluation of germplasm and breeding materials for heterotic groups in pearl millet.

Morphological characteristics and pedigree data have been utilised to define the germplasm employed in the creation of parental lines and open-pollinated varieties (Gupta et al., 2011). Nonetheless, physical characteristics are affected by environmental factors and do not adequately assess diversity. Evaluating genetic diversity, population structure, and linkage disequilibrium is essential for identifying heterotic groupings, implementing genomics-assisted breeding, and conserving resources. Understanding the population structure and genetic diversity of breeding populations, germplasm, and parental lines utilised in the breeding program is critically important for association mapping research, genomic selection, and genomics-assisted breeding.

Materials and Methods

Plant Materials

The experimental plant material comprised a diversified collection of twenty-four pearl millet inbreds, including eighteen Maintainer lines (females) and six Restorer lines (Testers), obtained from ICRISAT, Hyderabad (Table 1). This elite material was chosen during the Scientist's field-day selection. The selections were predicated on diversity to discover combiners exhibiting strong general and specific combining ability.

Table 1. Inbred Code, and type of the parental pearl millet inbred lines.

<u>S.No</u>	Female	<u>S.No</u>	Female	<u>S.No</u>	Female	<u>S.No</u>	Male
1	PMB103	7	PMB110	13	PMB124	1	PMR228
2	PMB104	8	PMB113	14	PMB125	2	PMR230
3	PMB105	9	PMB114	15	PMB133	3	PMR203
4	PMB106	10	PMB115	16	PMB134	4	PMR249
5	PMB108	11	PMB122	17	PMB137	5	PMR247
6	PMB109	12	PMB123	18	PMB142	6	PMR248
Total				18 Lines		6 Testers	

Production and Evaluation of F₁ Hybrids

The experiment was conducted during summer 2023 season, all possible line × tester crosses were made among the twenty four lines in order to obtain seeds of 108 F₁ hybrids. During the 2023 Kharif season, 108 F₁ hybrids were cultivated in the crossing block at the Rallis India Limited Research and Development Centre in Hyderabad, Telangana, India. All 108 F₁ hybrids, in conjunction with the commercial check hybrid MP7878 and their corresponding progenitors, were assessed utilising a Randomised Complete Block Design (RCBD). The experimental material consisted of four-row plots, each 4.8 m long, with a row spacing of 0.70 m and a plant-to-plant distance of 15 cm. Following the germination of the planting material, thinning was done at 12-15 days, followed by weeding at 20-25 days, and earthing up between 30-35 days to optimise growth and production. Nitrogen (N) was applied at 50 to 60 kg/ha, phosphorus (P) at 50 kg/ha, and potassium (K) at 20 to 30 kg/ha; further modifications can be done according to soil nutrient concentrations. These nutrients facilitate the growth of vigorous plants and augment overall yield. All agronomic practices were followed to raise a good crop.

Data Collection

Data was collected on five randomly selected plants over three replications of both parents and hybrids for 13 distinct quantitative attributes, namely: days to 50% Flowering (DTF) which is noted when fifty percent of the panicles on a plant have emerged, recorded in days. Plant Height (PH) is measured in centimeter from the base to the apex of the tallest leaf or panicle. Panicle Length (PL) is observed in centimeter from the base to the apex, whereas Panicle Girth (PG) is measured in centimeter around the broadest section of the panicle. The count of Productive Tillers per Plant (PTPP) includes only those with mature panicles, whereas Leaf Length (LL) and Leaf Width (LW) were recorded in centimeter at their maximum length and width, respectively. Head Weight per Plot (HW) denotes the weight in

kgs of harvested grain heads, whereas Grain Yield per Plot (GY) signifies the total mass of grains collected post-threshing, also measured in kgs. Single Panicle Weight (SPW) quantifies the weight in grams of a singular panicle, whereas Single Panicle Grain Yield (SPGY) assesses the grain yield in grams derived from a single panicle post-threshing. The 1000-grain Weight (TGW) denotes the weight in grammes of grains, whereas Fodder Yield per Plot (FYLD) refers to the biomass collected for fodder, quantified in kgs following a 3-day sun-drying phase.

Molecular Analysis

DNA Isolation

Leaves were sampled from 10 to 15 seedlings of each inbred line after twenty days from planting. Genomic DNA was isolated using CTAB method. Doyle, J. et.al. (1991) DNA quantity as well as quality was assessed using NanoDrop spectrophotometer (ND-1000, USA).

SSR Primers and PCR Amplification

Of the twenty-two SSR primer pairs examined, fourteen exhibited polymorphism among the twenty-four inbred lines investigated (Table 5). SSR markers encompassing the pearl millet genome were chosen from the literature to examine the diversity among the genotypes (Patil et al., 2021; Verma et al., 2021). The markers examined and their polymorphism information are shown in Table 2. The polymerase chain reaction (PCR) was conducted in a volume of 10 µl. The reaction mixture comprised 1µl of 10X Taq buffer A, 1µl of 2.5mM dNTPs, 1µl of oligonucleotide primers (both forward and reverse), 1µl of 1.2% BSA, 0.5µl of 1.5mM MgCl₂, 2µl of 50ng genomic DNA, 0.1µl of 5U/µl Taq DNA polymerase (Bangalore Genei Merck), and 3.4µl of autoclaved double-distilled water. The PCR was performed with the Eppendorf Master X50 PCR Thermal Cycler, USA. The PCR profile comprised an initial denaturation at 94 °C for 4 minutes, followed by denaturation at 94 °C for 30 seconds, annealing at 58 °C for 45 seconds (with annealing temperatures standardised for each SSR primer between 54 and 58 °C), an extension step at 72 °C for 1 minute, and

concluded with a final termination at 72 °C for 5 minutes, followed by a hold at 10 °C for 10 minutes. The amplified PCR product was separated on a 3% agarose gel (superfine resolution agarose, LonzaSeaKem). The gels were stained with ethidium bromide, subsequently destained with tap water, and photographed utilising a gel documentation system (BIO-RAD California, U.S.).

Table 2. Minimum, maximum and mean values of all the studied traits

Trait	Parameter	Value	Hybrid	Hybrids selected against the check
DTF	Minimum	51.00	PMB103×PMR228	83
	Maximum	61.67	PMB105×PMR228	
	Mean	55.51		
PH	Minimum	107.67	PMB103×PMR228	18
	Maximum	182.67	PMB110×PMR203	
	Mean	149.29		
PL	Minimum	20.67	PMB110×PMR230	17
	Maximum	28.00	PMB104×PMR228	
	Mean	24.17		
PG	Minimum	11.00	PMB142×PMR228	63
	Maximum	14.67	PMB123×PMR249	
	Mean	12.93		
PL	Minimum	1.67	PMB109×PMR249	18
	Maximum	6.33	PMB103×PMR247	
	Mean	3.08		
LL	Minimum	50.67	PMB108×PMR203	79
	Maximum	74.00	PMB142×PMR247	
	Mean	62.18		
LW	Minimum	3.13	PMB106×PMR228	81
	Maximum	6.00	PMB133×PMR248	
	Mean	4.36		
HW	Minimum	0.31	PMB103×PMR230	5
	Maximum	1.60	PMB105×PMR230	
	Mean	0.84		
GY	Minimum	0.20	PMB124×PMR247	4
	Maximum	1.19	PMB123×PMR247	
	Mean	0.64		
SPW	Minimum	28.85	PMB137×PMR230	56
	Maximum	109.33	PMB125×PMR249	
	Mean	71.32		
SPGY	Minimum	26.00	PMB103×PMR228	37
	Maximum	78.73	PMB125×PMR249	
	Mean	46.61		
SW	Minimum	8.23	PMB133×PMR249	23
	Maximum	15.97	PMB123×PMR203	
	Mean	13.03		
FYPP	Minimum	0.62	PMB133×PMR203	2
	Maximum	2.25	PMB106×PMR230	
	Mean	1.56		

Table 5: Correlation coefficients among parental genetic distance (GD), F₁ hybrid performance and SCA for all studied traits

Paramete r	DT F	PH	PL	PG	PL	LL	LW	HW	GY	SPW	SPG Y	TSW	FY
$r(\text{GD}, \text{F}_1)$	0.12	0.77*	0.32*	0.20*	-	0.40*	-	0.35	0.47*	0.42*	0.61*	-0.057	-
	*	*	*		0.24	*	0.04	*	*	*	*		0.14
					1		2						6
$r(\text{GD}, \text{SCA})$	0.00	0.03	0.04	-0.02	0.03	0.02	0.00	-	0.00	0.02	0.00	-0.05	0.04
								0.01					
$r(\text{F}_1, \text{SCA})$	0.00	0.03	0.01	0.00	-0.04	0.02	0.00	0.04	0.05	0.03	0.05	-0.01	-0.02

** Significant at 0.01 level of probability

Statistical Analysis

Analysis of variance (ANOVA) was conducted using all data utilising R software (version 4.4.1). The general combining ability (GCA) effects of the parents and the specialised combining ability (SCA) effects of the hybrids, along with their mean squares, were calculated using Griffing's method 4 model I (Griffing, B. et al., 1956) via the DiallelAnalysisR –R program. The significance assessment of GCA and SCA effects was conducted at 5% and 1% probability levels. Pearson's correlation coefficients (r) were computed and visualised utilising the corplot program by Wei, T. et al. (2019).

SSR Data Analysis

The amplified bands were evaluated for each SSR marker according to the presence or lack of bands, resulting in a binary data matrix of (1) and (0) for each marker. The scoring of the SSR alleles was conducted manually by assessing the position of the bands in relation to the 100bp ladder, progressing sequentially from the smallest to the largest bands. The null allele was reconfirmed. Bands identified as artefacts, diffused, exceedingly faint, or exhibiting null alleles, even

after reconfirmation, were excluded from the study. SSR bands were recorded as present (1) or absent (0) for each cultivar. The polymorphic information content (PIC) of each SSR marker was assessed according to the methodology outlined by Botstein et al. (1980).Genetic distances between pairs of inbred lines were computed based on Jaccard, P. et al. (1908), utilising the PAST software. The dendrogram was constructed using the unweighted pair group technique with arithmetic averages (UPGMA) in DARwin version 6.0.021.

Results

Combining ability analysis

Analysis of variance

For each studied trait, the analysis of variance (ANOVA) showed significant mean squares for parents, crosses, parents v/s crosses, lines, testers, and line × testers (Supplementary Table S1).

Table 1. Analysis of variance for combining ability, variance components and proportional contribution of quantitative traits in pearl millet

Parameters	DF	DIF	PH	PL	PG	PL	LL	LW	HW	GY	SPW	SPGY	TSW	FYLD
Replications	2	48.19**	19658.84**	47.93**	23.77**	7.02**	754.04**	7.11**	0.12**	0.15**	2044.16**	2892.80**	2.41ns	0.24**
Treatments	131	30.53**	3002.96**	23.73**	2.03**	1.12**	217.98**	0.76**	0.28**	0.19**	1025.22**	478.44**	8.86**	0.28**
Parents	23	54.01**	1263.32**	37.13**	1.87**	1.56**	97.69**	0.90**	0.07**	0.04**	377.06**	138.14**	17.91**	0.24**
Parents vs. Crosses	1	999.38**	305463.28**	1395.58**	37.50**	0.73ns	16941.38**	9.89**	9.60**	6.67**	30979.60**	21574.05**	241.71**	0.14ns
Crosses	107	16.43**	550.17**	8.03**	1.73**	1.03**	87.55**	0.65**	0.24**	0.17**	884.60**	354.44**	4.74**	0.29**
Lines	17	38.98**	1338.79**	23.99**	2.61**	2.50**	242.00**	1.52**	0.58**	0.42**	2518.59**	780.77**	13.92**	0.52**
Testers	5	38.00**	1677.99**	10.77ns	8.82**	0.49ns	114.85ns	2.91**	0.37ns	0.30ns	4985.12**	2362.18**	5.57ns	0.73**
Lines X Testers	85	10.65**	326.10**	4.68**	1.13**	0.77**	55.05**	0.34**	0.17**	0.11**	316.60**	151.07**	2.85**	0.22**

With the exception of productive tillers and fodder output per plot, the mean square of the "parents v/s crosses" was significant for every characteristic under study. Tester effects were found to be significant for all traits except panicle length, productive tillers, leaf length, head weight per plot, grain yield, and thousand seed weight, indicating the prevalence of additive genetic effects (mean squares). The concurrent importance of mean squares attributable to lines, testers, and their interaction for days to flowering, plant height, panicle girth, leaf width, single panicle weight, single panicle grain weight and fodder yield per plot suggests that both additive and non-additive gene actions are implicated in the genetic regulation of these traits. These results corroborate previous studies by Dangariya et al. (2009), Shinde (2011), Rai et al. (2012), Govindraj et al. (2013), Khandagale et al. (2014), and Gavali et al. (2018).

Performance of F_1 Hybrids

In assessing the performance of 108 F_1 hybrids and the commercial check hybrid MP7878, several critical features were examined, demonstrating considerable diversity. The days to 50% flowering (DTF) indicated an early flowering period of 51.00 days for the hybrid PMB103×PMR228 and a maximum of 61.67 days for PMB105×PMR228, resulting in a mean of 55.51 days. A total of 83 hybrids surpassed the check for this feature. Plant height (PH) ranges from 107.67 cm (PMB103×PMR228) to 182.67 cm (PMB110×PMR203), with an average height of 149.29 cm, with 18 hybrids exceeding the control. Panicle length (PL) exhibited a minimum of 20.67 cm (PMB110×PMR230) and a maximum of 28.00 cm (PMB104×PMR228), yielding a mean of 24.17 cm, with 17 hybrids surpassing the control.

Panicle Girth (PG) varied between small 11.00 cm (PMB142×PMR228) and big 14.67 cm (PMB123×PMR249), with a mean of 12.93 cm, and 63 hybrids exceeded the check. Productive Tillers (PT) were found in the range between 1.67 tillers /plant (PMB109×PMR249) to 6.33 tillers /plant (PMB103×PMR247), with a mean of 3.08 tillers per plant and 18 hybrids did better than the check. Leaf Length (LL) ranged from short 50.67 cm (PMB108×PMR203) to long 74.00 cm

(PMB142×PMR247), averaging 62.18 cm, with 79 hybrids outperforming the check. Leaf Width (LW) ranged between 3.13 cm (PMB106×PMR228) to 6.00 cm (PMB133×PMR248), with a mean of 4.36 cm, and 81 hybrids performed better than the check. Head Weight /Plot (HW) ranged from 0.31 kg (PMB103×PMR230) to 1.60 kg (PMB105×PMR230), averaging 0.84 kg, with 5 hybrids surpassing the check. Grain Yield /plot (GY) showed a minimum of 0.20 kg (PMB124×PMR247) and a maximum of 1.19 kg (PMB123×PMR247), with a mean of 0.64 kg. The maximum, minimum and mean values for all the studied traits are presented in Table 2.

The Single Panicle Weight (SPW) ranged from a minimum of 28.85 g (PMB137×PMR230) to a maximum of 109.33 g (PMB125×PMR249), with an average of 71.32 g, and 56 hybrids surpassed the control. Single Panicle Grain Yield (SPGY) varied from a minimum of 26.00 g (PMB103×PMR228) to a maximum of 78.73 g (PMB125×PMR249), with an average of 46.61 g, with 37 hybrids above the control. The 1000 Seed Weight (TSW) ranged from a minimum of 8.23 g (PMB133×PMR249) to a maximum of 15.97 g (PMB123×PMR203), with an average of 13.03 g, and 23 hybrids were selected in comparison to the control.

The results on Fodder Yield /plot (FYPP) ranged between 0.62 kg (PMB133×PMR203) to 2.25 kg (PMB106×PMR230), with a mean of 1.56 kg, and 2 hybrids outperformed the check MP7878. These results indicate that a substantial number of hybrids demonstrated superior performance compared to the check for various traits, highlighting the potential for selecting improved hybrids in breeding programs.

General Combining Ability (GCA) Effects

In the assessment of lines and testers for General Combining Ability (GCA), various lines and testers exhibited significant values. Multiple lines (PMB 109, PMB 103, PMB 142) and the tester PMR 203 exhibited considerable negative GCA effects, demonstrating their potential for early blooming. Lines PMB 125, PMB 133, and PMB 142 demonstrated significant GCA effects on yield and its associated

characters, including plant height, panicle length, productive tillers, head weight, grain yield, single panicle weight, single panicle grain yield, 1000-grain weight, and fodder yield. Among testers, PMR 247 and PMR 249 had substantial positive GCA effects for the majority of yield-related variables. These findings underscore their potential in breeding efforts aimed at enhancing early blooming and production. Various estimates of GCA are presented in Supplementary Table S2 and S3.

Table 2. General and specific combining ability variance components for quantitative traits in Pearl millet

Source	DT	PH	P	PG	PL	LL	LW	HW	GY	SPW	SPGY	TSW	FYLD
GCA variance	1.57405	56.20645	1.07284	0.682206	0.096685	10.38592	0.065328	0.027609	0.017356	122.3529	34.98344	0.015172	0.016643
SCA variance	0.596633	25.03493	0.11283	0.142262	-0.00516	1.307456	0.047611	0.003777	0.003567	86.45409	40.94656	0.05106	0.009541
GCA/SCA	8.10083	2.247278	9.50848	0.577872	-18.7418	9.87178	1.572113	6.113272	4.880584	1.413064	0.834368	12.21561	1.744338

Lines	DT	PH	P	PG	PL	LL	LW	HW	GY	SPW	SPGY	TSW	FYLD
PMB103	-2.224**	-21.954**	-1.009*	-0.576**	1.028**	-4.708**	-0.356**	-0.225**	-0.183**	-17.299**	-13.779**	0.144	-0.692*
PMB104	-0.951*	-8.009**	0.040	-0.348	0.689**	-5.792**	-0.119	-0.226**	-0.181**	-15.821**	-11.943**	-0.44	-0.195**
PMB105	1.772**	2.88	1.105*	-0.515*	-0.139	0.292	0.136	0.056	0.012	3.234	1.613	0.436	-0.109**
PMB106	1.049*	11.249**	-1.249**	-0.208	0.306*	-3.403**	-0.447**	0.105*	0.081*	7.346*	2.724*	0.522	0.116**
PMB108	1.772**	-5.026*	-0.284	-0.376	0.136	-5.542**	-0.447**	-0.231**	-0.182**	6.012	0.724	0.355	-0.183**
PMB109	-1.849**	8.546**	0.383	-0.153	-0.306*	-4.486**	-0.103	-0.014	-0.016	1.623	-1.331	-1.081**	-0.124**
PMB110	-0.764	10.249**	-1.451**	0.208	-0.139	1.097	-0.014	-0.152**	-0.131**	5.846	8.335**	0.31	-0.190**
PMB113	-0.596	3.933*	1.160**	0.097	0.194	4.764**	0.197*	-0.140**	-0.099**	-5.821	-5.998**	0.372	0.013
PMB114	1.169*	-2.843	-1.173**	0.524*	-0.528**	0.485	0.003	-0.150**	-0.123**	3.234	-1.165	-0.095	-0.310**
PMB115	0.549	-4.676	-2.349**	0.152	-0.361*	-0.681	0.258**	0.043	0.041	-0.432	-2.220*	0.046	0.142**
PMB122	3.105**	0.88	0.549	0.33	-0.194	2.597*	0.281**	0.078*	0.045	25.123**	12.557**	-0.073*	0.05
PMB123	-0.173	5.991*	0.549	0.541*	-0.139	0.431	-0.064	-0.120**	-0.104**	1.679	-0.943	1.832**	0.170**
PMB124	-0.895*	3.713	1.272**	0.263	0.136	-2.569*	0.369**	-0.097	-0.031	-0.488	-2.831*	1.103**	0.212**
PMB125	0.16	-3.065	-0.784	0.658*	-0.026	0.985	0.075	0.142**	0.116**	9.461**	3.691**	0.538	0.242**
PMB133	-0.006	5.491*	0.883*	0.291	-0.028	4.042**	0.036**	0.421**	0.347**	17.568**	9.333**	-2.201**	-0.043
PMB134	0.549	-0.221	2.105**	0.208	-0.25	2.993**	-0.175	0.088*	0.079*	2.061	-2.109	-0.517	-0.005
PMB137	-0.117	-10.509**	0.16	-0.487*	-0.063	4.264**	-0.175	0.111**	0.043	-17.156**	0.335	-0.595*	0.250**
PMB142	-2.617**	3.933	0.16	-0.32	-0.25	3.319**	-0.125	0.262**	0.297**	-18.651**	3.602*	-0.112	0.048

Table3. Proportional contribution of quantitative traits towards variance in Pearl millet

Source	DT	PH	P	PG	PL	LL	LW	HW	GY	SPW	SPGY	TSW	FYLD
Contribution of line %	37.70106	38.66167	47.47046	24.03059	38.68282	43.91718	37.12182	38.31884	39.92002	45.23504	34.99841	46.70618	28.46863
Contribution of tester %	10.81127	14.25207	6.267786	23.8393	2.212129	6.130397	20.94515	7.163871	8.410223	26.33383	31.14291	5.494883	11.8744
Contribution of line x tester %	51.48767	47.08625	46.26175	52.13011	59.10506	49.95242	41.93303	54.51729	51.66976	28.43112	33.85868	47.79894	59.65697

*Significant at 5% level. ** significant at 1% level

The research revealed that the variance attributed to general combining ability (σ^2_{gca}) exceeded that of specific combining ability (σ^2_{sca}) across all examined traits, suggesting that additive gene activity primarily governs these characteristics. Additive genetic variation exceeded dominant genetic variance for all variables, as seen by the ratio of general to specific combining ability variance ($\sigma^2_{gca}/\sigma^2_{sca}$), which was greater than one.

Specific Combining Ability (SCA) Effects

Numerous crosses demonstrated negative and significant Specific Combining Ability (SCA) effects for days to 50% flowering at the 1% level of significance. The estimates of SCA values are presented in Supplementary Table S4.

Table 4. Estimates of general combining ability effects for lines and Testers

Testers	DTF	PH	PL	P	PL	LL	LW	HW	GY	SPW	SPGY	TGW	FY
PMR203	-1.265**	4.435*	0.086	0.217	-0.083	-0.782	-0.056	0.004	-0.001	-0.587	0.017	-0.128	-0.153**
PMR228	0.049	-3.917*	-0.006	-0.22	-0.12	-0.745	-0.131*	-0.052	-0.037	-4.046	-3.280**	-0.543*	-0.058*
PMR230	-0.043	-7.435**	-0.321	-0.576**	0.139	-1.977*	-0.375**	-0.115**	-0.101**	13.056**	-8.739**	0.023	-0.047
PMR247	0.660*	-2.25	0.716*	0.215	0.065	0.412	0.223**	0.129**	0.125**	3.731	2.624**	0.255	0.149**
PMR248	-0.506	1.583	-0.599*	-0.19	0.009	0.995	0.140*	0.042	0.026	-2.052	-1.665*	0.372	0.128**
PMR249	1.105**	7.583**	0.123	0.554**	-0.009	2.097*	0.199*	-0.007	-0.012	16.009**	11.043**	0.022	-0.018
SE line	0.40028	2.516204	0.381618	0.195297	0.143144	0.907523	0.086316	0.036959	0.031048	3.213168	1.048704	0.27193	0.034903
SE tester	0.231102	1.452731	0.220327	0.112755	0.082644	0.523959	0.049835	0.021338	0.017926	1.855124	0.605469	0.156999	0.020151
S.E. (gl - g)line	0.566081	3.55845	0.53969	0.276192	0.202436	1.283432	0.12207	0.052267	0.043909	4.544106	1.483091	0.384567	0.04936
S.E. (gl - g)tester	0.326827	2.054472	0.31159	0.159459	0.116876	0.74099	0.070477	0.030177	0.025351	2.623541	0.856263	0.22203	0.028498

The crosses comprise PMB114 x PMR230, PMB113 x PMR247, PMB105 x PMR248, PMB114 x PMR228, PMB133 x PMR249, and PMB115 x PMR230. These findings suggest that these particular crosses are potential prospects for the development of early-flowering hybrids. Among the top 5% of crosses exhibiting substantial and positive Specific Combining Ability (SCA) impacts for yield-related traits, several noteworthy combinations were identified. Regarding plant height, PMB110 x PMR203, PMB137 x PMR228, and PMB108 x PMR247 shown the most efficacy. Panicle length was positively affected by PMB104 x PMR228 and PMB109 x PMR249, whereas PMB124 x PMR228 had the greatest importance for panicle girth. The highest crosses for productive tillers per plant were PMB113

x PMR249 and PMB106 x PMR248. The most effective crosses for leaf length were PMB137 x PMR203, PMB104 x PMR228, and PMB142 x PMR247. These superior crosses are significantly valuable for breeding programs focused on improving specific yield-related features. The results demonstrate that these particular crosses are exceptionally effective in strengthening many features, rendering them beneficial for breeding programs aimed at improving yield and its associated qualities.

Cluster Analysis, Genetic Distance (GD) and SSR Polymorphisms

Of the twenty-two SSR primer pairs examined, fourteen exhibited polymorphism among the twenty-four inbred lines investigated (Table 3). The primer pairings produced a total of 42 polymorphic fragments (Figure 1).

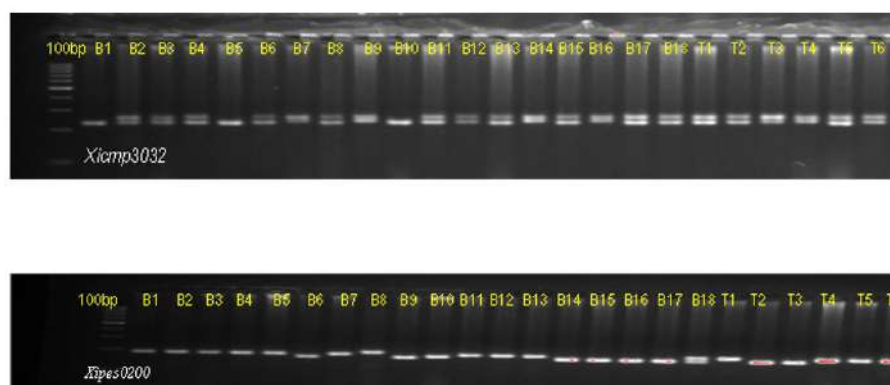


Figure 1. Amplification pattern of representative SSR markers with the eighteen females and 6 testers pearl millet inbred lines (B1–B18 & T1 –T6), with 100 bp DNA ladder.

The allele count per locus varied from 2 to 4, with a mean of 3 alleles per locus (Table 2). The polymorphic information content (PIC) was 0.561, with a range of 0.279 to 0.761. The *Xpsmp2079.2* locus exhibited the highest PIC of 0.761 (Table 3). Estimates of genetic distance derived from SSR markers varied from 0.164 to 0.569, with a mean of 0.366 (Supplementary Table S5).

Table 5. Estimates of specific combining ability effects of line x tester.

S.No.	Line x Tester	DFP	PLHT	PNLTH	PNGRTH	PDLR	LEAF L	LEAF W	HW	GV	SPW	SPGV	SEEDWT	YIELD
1	PMB1032P9.R228	-2.321*	-13.750*	-0.494	0.798	-0.657	-2.727	-0.264	0.031	0.015	-8.676	-1.554	1.271	-0.003**
2	PMB1032P9.R230	1.099	-9.231	-0.840	1.120*	-0.583	0.171	-0.053	-0.136*	-0.115	2.667	1.906	-0.362	-0.208
3	PMB1032P9.R233	1.321*	7.808	-0.92	-0.073	-0.361	-0.19	0.129	-0.085	-0.105	-12.138	-6.850**	0.923	-0.128**
4	PMB1032P9.R249	0.284	3.417	2.943*	-0.91	-0.435	1.264	0.14	0.233*	0.107*	9.602	2.124	-0.194	0.32
5	PMB1032P9.R247	-0.865	3.583	-0.215	-0.771	2.150**	-1.384	0.182	0.04	0.013	5.88	3.876	-1.427*	-0.08
6	PMB1032P9.R248	-0.772	8.063	0.432	-0.185	-0.12	2.866	-0.134	0.001	0.020	2.663	2.498	-0.21	0.151
7	PMB1042P9.R228	-1.272	-12.094*	3.784**	0.137	0.731*	6.023**	-0.047	0.035	-0.01	5.548	2.38	0.288	-0.04
8	PMB1042P9.R239	0.488	-6.843	-2.235*	-0.341	-0.194	1.921	0.007	0.025	0.03	2.558	3.072	-0.579	-0.044
9	PMB1042P9.R263	1.71	5.82	-0.975	-0.997*	-0.308	-1.308	-0.135	-0.087	-0.037	6.913	-3.017	0.373	0.068**
10	PMB1042P9.R249	-1.327	15.806*	-0.679	0.186	0.287	-0.486**	-0.444*	0.164	0.155*	-8.176	-6.043*	0.123	-0.244
11	PMB1042P9.R247	-1.549	-9.028	0.395	0.808	-0.12	-3.001	0.332	-0.185*	-0.150*	3.102	3.709	1.09	0.153
12	PMB1042P9.R248	1.851*	7.138	-0.29	0.107	-0.398	3.949	0.210	0.002	0.007	3.883	1.998	-1.494*	0.077**
13	PMB1052P9.R228	4.340**	-18.383**	-0.938	-0.697	-0.157	-4.001	0.131	-0.086	-0.04	-12.176	-8.270**	0.277	-0.182**
14	PMB1052P9.R230	-6.301	-15.065*	0.377	0.159	0.583	-3.495	-0.158	0.010**	0.017**	-7.5	-3.817	-0.957	0.3
15	PMB1052P9.R263	-2.348*	4.791	-1.498	0.7	0.139	-1.023	-0.244	-0.039	-0.033	14.031	11.428**	-0.205	0.040
16	PMB1052P9.R249	2.284*	9.583	0.599	-0.304	0.095	3.284	0.708**	-0.283**	-0.202**	0.769	0.009	0.745	-0.089
17	PMB1052P9.R247	-0.272	13.081*	0.006	-0.132	-0.543	5.449*	-0.156	-0.137	-0.162*	4.58	2.487	0.845	-0.116
18	PMB1052P9.R248	-3.103**	6.25	1.854	0.274	-0.287	-0.134	-0.14	-0.270**	-0.150*	0.496	-1.891	-0.705	0.148
19	PMB1062P9.R228	0.393	4.381	-0.18	-0.413	-0.602	-3.500	-0.655**	-0.042	-0.003	1.648	0.280*	0.80	0.069**
20	PMB1062P9.R230	1.488	7.233	-0.512	0.106	-0.528	-1.408	-0.142	0.534**	0.225**	9.389	6.972*	0.15	0.021**

S.No.	Line x Tester	DFP	PLHT	PNLTH	PNGRTH	PDLR	LEAF L	LEAF W	HW	GV	SPW	SPGV	SEEDWT	YIELD
21	PMB1082P9.R263	-0.29	-7.324	-0.233	-0.85	0.028	8.005**	-0.127	-0.164	-0.125	-14.413	-9.088**	-0.022	-0.320**
22	PMB1082P9.R249	-2.337*	1.184	-0.957	0.646	0.287	-3.208	0.118	-0.037	-0.003	-15.009	-13.708**	-1.105	-0.308
23	PMB1082P9.R247	0.451	1.381	1.117	0.652	-0.454	2.477	0.527*	0.084	0.053	19.602*	11.576**	0.095	0.025
24	PMB1082P9.R248	0.284	-6.800	0.705	0.057	1.269**	-2.44	0.277	-0.155	-0.134	-0.613	-0.335	0.012	-0.127**
25	PMB1082P9.R228	3.066**	-1.683	0.431	-0.335	-0.435	0.773	0.081	-0.12	-0.136	-1.954	1.946	0.76	0.434**
26	PMB1082P9.R230	-0.508	8.435	0.705	0.553	-0.028	0.671	0.392	-0.164*	-0.129	8.656	5.739*	0.46	-0.435
27	PMB1082P9.R263	-0.879	-11.102	1.358	0.727	0.394	-5.190*	0.373	-0.152	-0.099	2.253	0.317	0.345	-0.074**
28	PMB1082P9.R249	1.951*	-17.917**	-0.148	-0.11	-0.213	8.697**	-0.210	-0.155	-0.134	3.991	-4.943	0.102	-0.429**
29	PMB1082P9.R247	-1.272	16.250**	-1.605	0.063	0.046	-2.011	-0.14	0.000**	0.282**	-6.664	-3.291	-0.738	0.261**
30	PMB1082P9.R248	-2.438*	5.417	-0.623	-0.698	0.435	-0.561	-0.490*	0.303**	0.218**	-4.282	-3.659	-0.988	0.245
31	PMB1092P9.R228	0.491	1.083	-0.218	0.109	0.009	-1.782	-0.194	-0.180*	-0.129	-3.232	-2.805	-2.708**	-0.108
32	PMB1092P9.R230	0.71	-4.665	-0.901	0.465	0.750*	0.282	-0.180	-0.02	0.011	6.778	2.794	1.499*	-0.106
33	PMB1092P9.R263	0.599	-5.269	-1.975*	-0.661	0.659	0.688	-0.595*	0.661	0.034	3.975	-0.620	-1.345*	0.154*
34	PMB1092P9.R249	-1.438	0.383	2.684**	0.502	-1.102**	-5.125*	0.173	0.019	0.006	2.3	0.346	0.767	-0.218**
35	PMB1092P9.R247	-0.86	3.75	0.728	-0.326	-0.170	0.580**	0.349	0.078	0.009	-13.342	-4.509	0.407	0.272
36	PMB1092P9.R248	-0.16	5.917	-0.29	-0.087	-0.12	-0.023	0.332	0.05	0.068	5.44	4.72	1.384*	0.086
37	PMB1102P9.R228	-0.438	16.028*	1.284	0.415	0.176	6.134**	0.484	0.075	0.077	-0.454	-0.002	0.004	0.000**
38	PMB1102P9.R230	-0.340	-11.434	-1.735	-0.25	-0.417	-7.361**	-0.342	-0.136	-0.087	10.530	9.794**	0.038	0.389
39	PMB1102P9.R263	0.543	18.070**	1.191	0.477	0.472	-0.829	-0.194	0.190*	0.100*	-6.913	-7.951**	0.523	0.109*
40	PMB1102P9.R249	1.84	-7.472	0.154	-0.526	0.398	1.625	0.351	-0.08	-0.105	5.491	-0.907	0.14	-0.223

S.No	Line x Tester	DF	PLHT	PNLTH	PNGRTH	PDLR	LEAF L	LEAF W	HW	GY	SPW	SPGY	SEEDWT	YLD
41	PM0110XPM0247	0.951	-21.972**	-0.438	-0.187	-0.343	-0.356**	-0.04	-0.269**	-0.252**	-8.231	-7.902**	-0.894	-0.11
42	PM0110XPM0248	-2.549*	6.194	-0.457	0.052	-0.287	6.727**	6.61	0.215*	0.307**	-6.448	9.054	0.19	-0.065
43	PM0110XPM0228	-2.383*	10.301	-0.327	0.092	-0.824*	-2.532	-0.064	-0.047	-0.972	1.213	0.062	0.21	0.142
44	PM0110XPM0219	2.710**	5.85	0.654	0.048	-0.083	2.032	0.281	-0.195	-0.078	-7.111	-1.539	0.277	0.948**
45	PM0110XPM0203	1.599	-8.991	0.247	-0.078	0.139	-0.828	-0.071	-0.193*	-0.108*	-8.913	-3.294	-0.072	0.407
46	PM0110XPM0249	1.562	-14.806*	-0.123	-0.415	1.005**	0.292	-0.06	0.041	0.027	1.158	-0.587	0.012	-0.022**
47	PM0110XPM0247	-2.994**	-6.639	1.617	-0.243	-0.009	0.31	-0.251	0.242**	0.216**	14.436	8.098**	-0.588	-0.242**
48	PM0110XPM0248	-0.484	8.194	-2.068*	-0.004	-0.287	0.727	0.168	0.062	0.076	-0.782	-2.28	0.162	-0.33
49	PM0110XPM0228	-3.039**	8.806	0.673	0.155	0.585	0.079	-0.203	0.039	0.049	-1.843	-0.831	0.343	-0.083
50	PM0110XPM0219	-2.957**	3.324	-0.012	-1.047*	0.306	0.977	0.142	0.258**	0.222**	-8.853	-4.039	-0.723	0.906
51	PM0110XPM0203	-1.068	10.454	-0.065	0.494	-0.472	-1.318	-0.144	0.103	0.079	18.696*	11.339**	-1.905**	0.105
52	PM0110XPM0249	1.895	5.972	0.543	0.057	0.12	-4.704*	-0.066**	0.090	-0.059	4.769	1.18	2.078**	-0.042*
53	PM0110XPM0247	2.066*	-17.194**	-1.049	-0.337	-0.287	2.588	6.577**	-0.049	-0.057	-0.953	-0.402	0.212	-0.183*
54	PM0110XPM0248	3.113**	-11.581	-0.068	0.008	-0.231	2.338	0.294	-0.285**	-0.234**	-11.817	-7.446**	-0.003	0.158
55	PM0110XPM0228	-0.772	-8.494	-0.827	-0.53	0.005	-1.755	0.242	0.256**	0.198*	-8.176	-3.109	-0.029	0.903
56	PM0110XPM0219	-4.012**	7.824	-0.179	-0.008	0.472	-2.856	-0.147	0.029	0.012	4.854	7.683	0.238	-0.008
57	PM0110XPM0203	-1.457	2.62	-1.253	1.033*	-0.306	-2.384	0.068	0.200*	0.201**	24.384**	14.594**	-0.244	0.108*
58	PM0110XPM0249	2.566*	7.472	2.043*	0.696	-0.046	8.069**	0.379	0.048	0.07	13.789	9.902**	0.34	0.186*

S.No	Line x Tester	DF	PLHT	PNLTH	PNGRTH	PDLR	LEAF L	LEAF W	HW	GY	SPW	SPGY	SEEDWT	YLD
59	PM0115XPM0247	3.284**	-5.694	-0.216	-0.132	-0.484	-1.912	-0.222	-0.099	-0.063	-15.287	-11.012**	0.406	-0.187
60	PM0115XPM0248	0.401	-5.528	0.432	-1.060*	0.269	0.838	-0.329	-0.478**	-0.424**	-19.504*	-14.057**	-0.71	-0.100**
61	PM0115XPM0228	2.006*	-12.25	-1.049	0.459	0.331	0.654	0.119	-0.235*	-0.179**	-0.005	1.440	-1.440**	-0.455**
62	PM0115XPM0219	2.099*	-2.065	0.599	0.481	0.306	4.199	0.364	-0.413**	-0.306**	8.612	4.905	0.154	-0.059**
63	PM0115XPM0203	-2.032*	0.069	0.191	-0.478	-0.139	-10.429**	-0.855	0.239**	0.274**	1.473	2.817	-0.26	0.243**
64	PM0115XPM0249	-0.049	5.917	-2.846**	-0.062	-0.233	-3.208	-0.177	0.190*	0.156*	-6.453	-5.676*	1.156	0.501**
65	PM0115XPM0247	-1.272	7.083	1.502	-0.33	-0.207	2.144	0.199	0.174	0.148	-4.509	-5.457*	0.99	0.248
66	PM0115XPM0248	-0.772	1.25	1.563	-0.071	0.102	6.560**	-0.151	0.038	-0.092	0.94	0.385	0.408	0.083**
67	PM0115XPM0228	-0.049	-0.684	-0.283	-1.085*	0.176	-0.109	0.131	-0.149	-0.12	-4.954	-3.387	-0.751	0.249
68	PM0115XPM0219	-0.29	0.157	1.265	-0.063	-0.062	1.022	0.075	-0.1	-0.1	-2.611	-1.628	-0.751	0.041**
69	PM0115XPM0203	1.265	-0.046	-1.142	-0.109	0.472	3.038	-0.144	-0.308**	-0.250**	0.253	-2.017	1.234	-0.256
70	PM0115XPM0249	-0.105	2.806	0.154	0.64	-0.209	2.958	-0.066	-0.161	-0.125	16.324*	11.957**	0.684	-0.058**
71	PM0115XPM0247	0.34	9.639	-0.772	-0.021	-0.009	-3.356	-0.323	0.680**	0.591**	2.919	2.043	-0.483	0.305**
72	PM0115XPM0248	-1.16	0.139	-0.877	0.718	-0.287	-5.273*	0.327	0.057	0.064	-11.948	-6.669*	0.097	-0.3
73	PM0115XPM0228	-1.06	13.917*	-0.195	1.526**	0.065	4.001*	0.197	0.080	0.096	4.546	-0.499	-0.112	0.005
74	PM0115XPM0219	-1.508	2.769	1.877**	0.581	-0.028	-0.034	0.008	-0.085	-0.061	-7.777	-5.706*	0.221	0.052**
75	PM0115XPM0203	0.054	2.898	1.136	-1.353*	-0.472	4.171	0.659**	0.187*	0.173*	-15.58	-10.481**	0.04	-0.806
76	PM0115XPM0249	-1.382	4.417	-0.558	-0.748	0.12	0.292	-0.109	0.394**	0.324**	12.491	11.512**	-0.21	0.089
77	PM0115XPM0247	2.062*	-13.417*	-0.16	-0.343	-0.397	-6.356**	-0.29	-0.581**	-0.536**	3.769	3.598	-0.01	0.073

S.No	Line x Tester	DF	PLHT	PNLTH	PNGRTH	PDLR	LEAF L	LEAF W	HW	GY	SPW	SPGY	SEEDWT	YLD
78	PM0120XPM0248	1.895	-10.583	-2.178*	0.329	0.102	-5.273*	-0.375	-0.061	-0.007	2.532	1.554	0.073	0.09
79	PM0120XPM0228	1.284	-7.639	-0.049	0.331	0.065	-0.421	0.058	0.174	0.129	4.657	4.246	0.877	-0.01
80	PM0120XPM0219	1.643	1.88	0.599	0.02	0.472	6.144	0.333	-0.03	-0.068	-15.333	-11.228**	-0.857	0.012
81	PM0120XPM0203	-0.715	-1.657	-0.142	0.001	-0.639	-5.718*	0.184	-0.002	0.005	-1.802	-4.05	0.495	0.118**
82	PM0120XPM0249	-0.438	2.194	-0.512	0.097	-0.040	7.758**	0.199	-0.133	-0.102	12.602	17.391**	-0.822	-0.317
83	PM0120XPM0247	-0.594	7.361	0.502	0.093	-0.12	-4.245	-0.492*	-0.027	-0.033	-7.787	-10.724**	-0.080	0.116
84	PM0120XPM0248	-0.16	-2.139	-0.457	-0.532	0.289	2.505	-0.179	0.02	0.003	7.663	4.365	0.395	0.081**
85	PM0120XPM0228	0.117	0.806	-2.049*	-0.169	0.005	3.523	0.047	-0.013	-0.014	-0.843	0.602	0.682	0.361**
86	PM0120XPM0219	0.21	6.994	0.265	-0.647	0.472	2.421	-0.075	0.015	0.006	8.167	4.794	1.832**	0.353**
87	PM0120XPM0203	-0.001	5.787	2.191*	-0.106	0.361	-0.44	-0.26	-0.024	-0.018	-4.636	-2.961	-1.366*	-0.741**
88	PM0120XPM0249	-3.665**	-7.561	-0.846	0.224	-0.38	-4.586*	-0.549*	0.094	0.117	-16.898	-13.320*	-2.616**	0.311
89	PM0120XPM0247	1.84	8.472	0.562	0.229	-0.12	1.699	0.027	-0.102	-0.1	-7.02	-5.569*	1.384*	-0.028**
90	PM0120XPM0248	2.349*	-14.991*	-0.123	0.408	-0.398	-2.218	0.810**	-0.032	-0.031	19.829*	9.634**	0.634	-0.251**
91	PM0120XPM0228	1.228	15.194*	-0.938	-0.252	-0.18	-5.338*	-0.292	0.051	0.031	10.824	7.446**	-0.168	-0.330**
92	PM0120XPM0219	-0.346	5.046	-0.623	-0.73	0.026	0.56	0.019	0.15	0.111	-5.5	-2.761	-0.268	-0.234
93	PM0120XPM0203	-1.79	-8.157	1.658	0.644	-0.083	1.565	0.268	-0.088	-0.069	3.564	3.483	0.284	-0.142**
94	PM0120XPM0249	-2.506*	-7.972	-0.461	-0.66	0.136	0.153	0.145	-0.257**	-0.208**	-22.888**	-16.209**	0.601	0.353
95	PM0120XPM0247	-1.019	-7.159	-0.06	0.313	0.435	1.835	0.088	0.15	0.085	4.713	1.543	-0.366	0.123**

SSOs	Lines Traces	DEF	PLHT	PSLTH	PSRTH	PSLR	LEAF L	LEAF W	HW	GV	SPW	SPCV	SEEDWT	YIELD
96	PM011370P01211	-0.189	8.613	0.985	0.133	-0.176	1.121	-0.159	0.008	0.001	0.008	4.889*	0.017	0.141
97	PM011370P01212	-0.195	10.199**	0.673	0.142	0.12	0.968	0.406	0.011	0.009	22.880**	1.869	1.143	0.120*
98	PM011370P01213	-0.012	25.124**	1.854	-0.025	-0.106*	0.189	-0.047	-0.240**	-0.211**	-11.28	-0.724**	0.942	0.170**
99	PM011370P01213	1.21	-14.540*	-0.085	0.105	-0.15	6.061**	0.491	-0.001	-0.101	1.081	1.372*	0.095	0.207
100	PM011370P01214	-2.150*	-12.694*	-0.123	-0.102	0.343	-0.208	0.312	-0.247**	-0.261**	-11.238	-6.120*	-1.822**	-0.051**
101	PM011370P01217	0.284	-1.194	-1.718	0.007	0.285	-0.89	-0.112*	0.011	0.1	-6.95	1.431	-6.391	-0.280*
102	PM011370P01218	0.784	-6.020	-0.401	-0.587	0.324	-3.773	-0.161**	0.471**	0.119**	4.179	1.729*	-6.331	-0.1
103	PM011370P01218	-1.112	-10.900	0.015	-1.501**	0.287	-0.142	0.06	0.012	-6.988	-0.990**	0.16	-0.006*	
104	PM011370P01219	1.834	-18.120*	-1.012	-0.025	-0.838	1.144	-0.211	-0.374	-0.182*	3.389	-3.172	-1.273	-0.171**
105	PM011370P01219	1.277	8.145	0.18	0.005	0.583	6.181**	0.118	0.181*	0.118	1.111	1.172	0.212	0.340**
106	PM011370P01219	-1.994*	0.361	-0.79	0.668	-0.177	-4.284	-0.261	0.288**	0.170*	-4.002	2.103	0.295	0.170**
107	PM011370P01217	-0.149	2.694	0.264	0.107	0.102	6.081**	0.110	-0.370**	-0.081	5.021	6.769*	-6.303	-0.11
108	PM011370P01218	1.284	5.713	0.265	0.148	-0.178	-8.481**	0.117	0.007	-0.018	-1.708	-0.18	0.112	-0.009
S.E. (pooled)		0.90401	0.145416	0.05477	0.478178	0.100059	2.121969	0.211431	0.00133	0.070051	7.870623	1.500789	0.66609	0.083404
S.E. (adj - 12/2000)		1.30091	0.174038	1.121007	0.4790139	0.4027804	5.141711	0.296008	0.118128	0.107174	11.19074	1.803818	0.941993	0.112007

Utilising the genetic distance matrix, pairs were classified into low, medium, and high genetic distances, and the top 1% of pairs in each category were determined. For pairs with low genetic distance (≤ 0.3), the most proximate genetic links were identified between B113 and B114 (0.164), B124 and B133 (0.164), and B124 and B125 (0.212), with genetic distances spanning from 0.164 to 0.212. Medium genetic distance couples (0.31 - 0.45) demonstrated considerable genetic divergence, with the highest pairs being B106 and B113 (0.291), B108 and B113 (0.282), and B106 and B108 (0.293), with distances ranging from 0.282 to 0.293. Significant genetic divergence was observed among high genetic distance couples (> 0.45), namely between B142 and R203 (0.569), R203 and R248 (0.567), and B134 and R203 (0.548), with genetic distances spanning from 0.548 to 0.569.

The dendrogram created from genetic distance (GD) exhibited two primary clusters, distinctly separated into male and female groupings. The female group comprises three subclusters: Cluster 1 contains eleven inbreds, Cluster 2 contains five inbreds, and Cluster 3 contains two inbreds. The male group comprises two subclusters: Cluster 4, including four inbreds, and Cluster 5, consisting of two inbreds (Figure 2). Information is provided in Table 4.

Table 3. Number of alleles, PCR product size and polymorphic information content(PIC) of the fourteen SSR markers used in this study.

Marker	LG	Repeat motif	No of alleles	Size range (bp)	PIC
<i>Xpsmp2079.2</i>	7		3	117-179	0.761
<i>Xpsmp2220</i>	5	(GT)11	2	136-156	0.279
<i>Xpsmp2070</i>	3	(CA)25 (TA)6	3	204-278	0.666
<i>Xipes0220.1</i>	3		3	172-216	0.575
<i>Xipes0186</i>	4	(TTG)10	3	169-293	0.405
<i>Xpsmp2089</i>	2	(AC)15	3	117-159	0.517
<i>Xipes0200</i>	6	(GTAC)11	3	174-210	0.525
<i>Xpsmp2203</i>	7	(GT)18 imp.	3	351-389	0.609
<i>Xpsmp2068</i>	2	(AC)14	4	112-162	0.576
<i>Xicmp3032</i>	1	(GCT)8	2	202-217	0.387
<i>Xpsmp2086</i>	4	(AC)14	3	107-149	0.628
<i>Xpsmp2030</i>	1	(CA)11 (GA)10	4	102-160	0.631
<i>Xpsmp2081</i>	4		4	250-260	0.669
<i>Xpsmp2018</i>	6		2	110-125	0.628

Table 4. Dendrogram of the eighteen females and six testers pearl millet inbred lines constructed from SSR data using (UPGMA) according to Jaccard's coefficients using Darwins software.

CLUSTERS	Inbreds	No of inbreds	Main Group
Cluster-1	B105,B122,B113,B114,B115,B134,B123,B125,B124,B103,B133	11	Lines(Females)
Cluster-2	B110,B137,B108,B104,B106	5	Lines(Females)
Cluster-3	B109,B142	2	Lines(Females)
Cluster-4	R203,R247,R228,R230	4	Testers(Males)
Cluster-5	R248,R249	2	Testers(Males)
	TOTAL INBREDS PARENTS	24	

Association between Genetic Distance (GD), F₁ Hybrid Performance and SCA

The correlations between the genetic distances of pairs of inbred lines and the performance of F₁ hybrids were strongly associated with the F₁ hybrid performance throughout the analysed variables (Table 4, Figure 2A, B). The Plant height had a significant connection with Genetic Distance ($r = 0.77$), suggesting that genetic similarity among parental inbreds substantially affects the performance of F₁ hybrids. This strong correlation indicates that Genetic Distance is a dependable predictor of plant height, essential for enhancing plant architecture in breeding initiatives. Likewise, characteristics such as SPGY ($r = 0.61$) and GY ($r = 0.47$) exhibited moderate to strong relationships with GD, underscoring its effectiveness in forecasting essential agronomic effects.

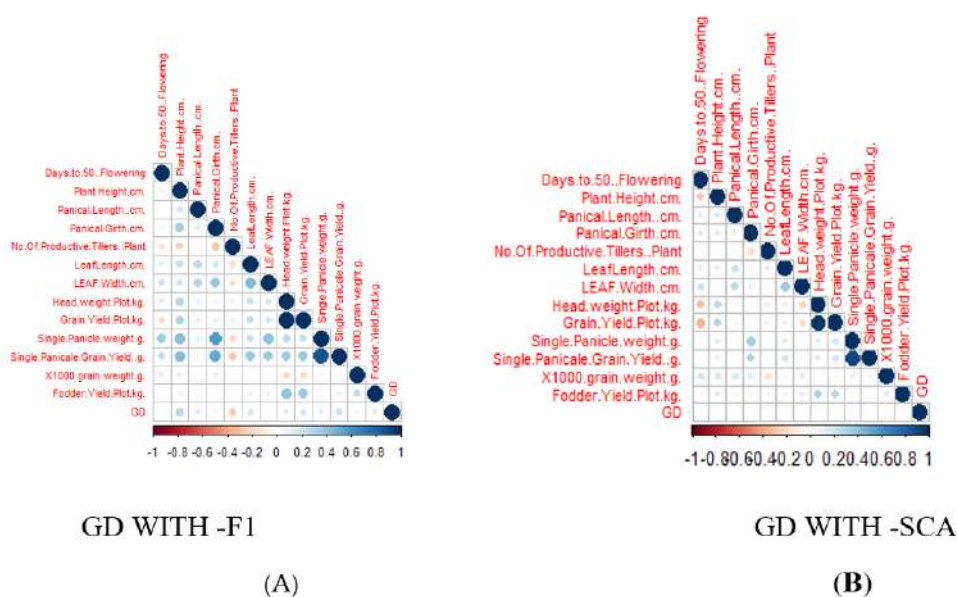


Figure 2. Corplot depicting correlation coefficient of genetic distance based on molecular data with F₁ hybrid performance (A) and SCA (B) for all studied traits

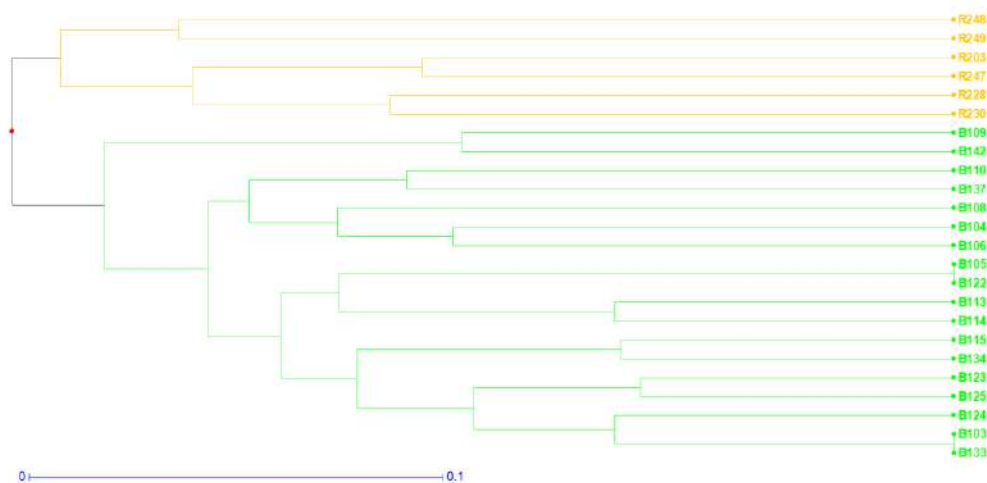


Figure 2. Dendrogram of the eighteen females and six testers pearl millet inbred lines constructed from SSR data using (UPGMA) according to Jaccard's coefficients using Darwins software.

In contrast, very weak correlations were noted for characters such as DTF ($r = 0.12$) and PDLR ($r = -0.241$). The results demonstrate that although GD may accurately forecast some features, its predictive efficacy fluctuates across several dimensions of plant development and production. Significantly, (TSW) demonstrated a negative connection with GD ($r = -0.057$), highlighting the intricacy of genetic determinants affecting seed traits.

DTF(Days to 50% Flowering), PH(Plant Height), PL(Panicle Length), PG(Panicle Girth), PT(Productive Tillers), LL(Leaf Length), LW(Leaf Width),HW(Head Weight), GY(Grain Yield),SPW(Single Panicle Weight), SPGY(Single panicle Grain Yield),TSW(1000 seed Weight), FY(Fodder Yield).

The present findings indicated a substantial association between molecular Genetic Diversity and F_1 hybrid performance (Singh, P et al., 2015; Betran, F et al., 2003). No significant association existed between genetic diversity (GD) and specific combining ability (SCA) across all traits, indicating that SSR-based GD may not

effectively predict SCA effects in the studied traits. A non-significant association between genetic distances and SCA was similarly reported by Makumbi, D et al. (2011) and Parentoni, S et al. (2001). However, in contrast to the current findings, Betran et al. showed a strong association between GD and SCA concerning maize grain yield. Moreover, our findings demonstrated that GD exhibits considerable promise in forecasting F₁ hybrid performance for particular features, suggesting that GD could be utilised to anticipate the performance of F₁ hybrids. This outcome aligns with the conclusions of Singh, P. et al. (2015) and Betran, F. et al. (2003).

Discussions

Analysis of Variance and Hybrid Performance

The mean squares for parents, parents v/s crosses, testers and line $\times$ tester interactions were highly significant in the analysis of variance (ANOVA) for the studied characteristics in pearl millet. This suggests that the hybrids exhibited considerable genetic variability, with both parents and crosses exhibiting significant effects on all traits. The prevalence of additive genetic effects in the inheritance of these traits is indicated by the significance of mean squares attributable to lines and lines $\times$ testers for all traits. This is essential for breeding programs that are dedicated to the enhancement of traits through the utilisation of additive genetic variation. The testers were effective in discriminating among lines for the majority of traits, but they were less effective for the exceptions of panicle length, productive tillers, leaf length, head weight per plot, cereal yield, and thousand seed weight.

The associated importance of mean squares attributed to lines, testers, and their interaction for traits including days to flowering, plant height, panicle girth, leaf width, single panicle weight, single panicle grain weight, and fodder yield per plot suggests the influence of both additive and non-additive genetic mechanisms in the regulation of these traits. The results align with earlier research conducted by Dangariya et al. (2009), Shinde et al. (2011), Rai et al. (2012), Govindraj et al. (2013), Khandagale et al. (2014), and Gavali et al. (2018), all of which emphasised

the significance of both categories of gene effects in the inheritance of agronomic characteristics.

The assessment of 108 F₁ hybrids compared to the commercial check hybrid MP7878 in pearl millet exhibited considerable heterogeneity across all examined parameters, indicating significant genetic diversity and the potential for choosing superior hybrids in breeding programs. Numerous hybrids surpassed the best check MP7878 across various traits, including days to 50% flowering (DTF), plant height (PH), panicle length (PL), panicle girth (PG), productive tillers (PT), leaf length and width (LL and LW), head weight per plot (HW), grain yield per plot (GY), single panicle weight (SPW), single panicle grain yield (SPGY), 1000 seed weight (TSW) and fodder yield per plot (FYPP).

The predominance of general combining ability (σ^2_{gca}) over specific combining ability variance (σ^2_{sca}) for these measures highlighted the significance of additive genetic factors in governing their inheritance. This tendency was uniform across characteristics such as plant height, panicle girth, leaf dimensions, and grain yield, wherein additive genetic variance surpassed dominant genetic variance. These findings substantiate the effectiveness of selective methods of breeding to improve these features in pearl millet. Furthermore, the contributions of parental lines and their combinations (line tester) differed among traits; for example, lines had a more substantial impact on traits such as panicle length and thousand seed weight, while line tester interactions were pivotal for traits like days to flowering and grain yield.

This study highlights the genetic diversity among F₁ hybrids and their potential for agronomic improvement in pearl millet breeding. The identification of hybrids that consistently surpass the commercial check across various traits presents appealing possibilities for additional evaluation and further introduction as new commercial cultivars. These findings aligns with the results published in earlier research by Dangariya et al. (2009), Rai et al. (2012), Govindraj et al. (2013), Khandagale et al. (2014), Gavali et al. (2018), and Ejigu et al. (2017).

SSR Polymorphisms, Genetic Diversity, and Cluster Analysis

The average number of alleles (2.7) per locus identified in this investigation was comparable to the values reported by Badu-Apraku et al. (2013), Akinwale et al. (2014), and Menkir et al. (2004), who observed averages of 2.9, 2.57, and 3.0 alleles per locus, respectively. Nonetheless, it was inferior than the 6.21 alleles/locus documented by Oppong et al. (2014) and the 5.7 alleles/locus identified by Oyekunle et al. (2015) in maize inbred lines utilising SSR markers. The variations in allele values between research may be ascribed to discrepancies in sample size, repeat length, and the quantity of SSR markers utilised, as noted by Akinwale et al. (2014). The reduced values noted in this study may result from the limited number of lines utilised in genotyping.

The genomic analysis of twenty-four inbred pearl millet lines utilising SSR markers offers critical insights into the genetic diversity and interrelationships among these lines. Of the twenty-two SSR primer pairs evaluated, fourteen exhibited polymorphism, resulting in a total of 42 polymorphic fragments. This signifies a moderate degree of genetic variation among the examined inbred lines, with each SSR locus containing 2 to 4 alleles and an average of 3 alleles per locus.

The polymorphic information content (PIC) of the markers varied from 0.279 to 0.761, with a mean PIC of 0.561, signifying a considerable degree of informativeness of the SSR markers in differentiating genotypes. The *Xpsmp2079.2* marker on linkage group 7 displayed the highest PIC value of 0.761, indicating its significant efficacy in identifying genetic diversity. Additional markers with elevated PIC values comprised *Xpsmp2070* (0.666), *Xpsmp2081* (0.669), and *Xpsmp2030* (0.631), which are crucial for the genetic mapping and marker assisted selection in pearl millet breeding.

The SSR markers enabled the assessment of genetic distances among the inbred lines, which varied from 0.164 to 0.569, with a mean genetic distance of 0.366. These distances indicate the extent of genetic divergence or similarity between pairs

of lines, which is essential for comprehending the breeding potential and relatedness among genotypes. Pairs were classified into low (≤ 0.3), medium (0.31 - 0.45), and large (> 0.45) genetic distances. The nearest genetic affiliations were noted between B113 and B114 (0.164), B124 and B133 (0.164), and B124 and B125 (0.212), with genetic distances varying from 0.164 to 0.212. These pairs have minimal genetic distance, signifying a high level of relatedness, which may be detrimental for hybrid breeding due to restricted heterotic potential.

Pairs with medium genetic distance, including B106 and B113 (0.291), B108 and B113 (0.282), and B106 and B108 (0.293), demonstrated moderate genetic divergence, advantageous for breeding programs aiming to optimise genetic diversity and performance. Pairs exhibiting high genetic distance (> 0.45), including B142 and R203 (0.569), R203 and R248 (0.567), and B134 and R203 (0.548), demonstrated considerable genetic divergence, rendering them viable candidates for hybrid breeding owing to their potential for substantial heterosis.

The dendrogram generated by the UPGMA method using DARwin software version (6.0.021) based on genetic distance displayed two primary clusters, distinctly segregated into male and female groups. The female group comprised three subclusters: Cluster 1 containing eleven inbreds, Cluster 2 consisting of five inbreds, and Cluster 3 with two inbreds. The male cohort had two subclusters: Cluster 4, consisting of four inbreds, and Cluster 5, comprising two inbreds. This clustering pattern signifies both shared ancestry and genetic divergence among the inbred lines, which is crucial for enhancing breeding tactics to leverage heterosis and develop improved cultivars.

The study emphasises the significance of utilising genetic variety, combining ability, and molecular markers to enhance breeding techniques and attain sustainable advancements in pearl millet output and adaptability.

Association between Genetic Distance, F1 Hybrid Performance and SCA

The recent findings indicate a substantial association between molecular GD and F₁ hybrid performance (Singh, P et al., 2015; Betran, F et al., 2003). No substantial association existed between GD and SCA across all characteristics, indicating that SSR-based GD may not effectively predict SCA effects in the examined traits. A non-significant connection between genetic distances and SCA was similarly reported by Makumbi, D et al. (2011) and Parentoni, S et al. (2001). Contrary to the current findings, Betran et al. (2003) observed a strong association between GD and SCA for higher grain production. Moreover, our findings revealed that Genetic Distance (GD) possesses considerable potential in forecasting F₁ hybrid performance for particular traits, suggesting that GD might be utilised to anticipate the performance of F₁ hybrids. This outcome aligns with the conclusions of Singh, P. et al. (2015) and Betran, F. et al. (2003).

Our findings demonstrated a significant correlation between genetic diversity (GD) and F₁ hybrid performance across the analysed traits, with plant height (PH) displaying a notably strong correlation with GD ($r = 0.77$), suggesting that genetic similarity among parental inbreds profoundly affects the stature of F₁ hybrids. This strong correlation indicates that GD is a dependable predictor of plant height, essential for enhancing plant architecture in breeding initiatives. Likewise, characteristics such as Single Panicle Grain Weight (SPGY) ($r = 0.61$) and Grain Yield (GY) ($r = 0.47$) exhibited moderate to substantial relationships with GD.

There were, however, weaker associations found for characteristics such as the number of days till flowering (DTF) ($r = 0.12$) and the number of productive tillers (PT) ($r = -0.241$). Based on these findings, it can be concluded that genetic diversity (GD) has the ability to accurately predict some characteristics; nevertheless, its predictive capacity differs across many elements of plant growth and production. It is important to note that the seed weight (SW) of 1000 seeds showed a negative correlation with the genetic diversity ($r = -0.057$), which highlights the complexity of the genetic processes that influence seed characteristics. Overall, while GD demonstrates significant potential in predicting F₁ hybrid performance for specific traits, This indicated that GD could be used to predict the performance of F₁ hybrids.

This result are in agreement with the findings of Singh, P. et.al.(2015), Betran, F. et. al (2003). These findings contribute valuable insights into the intricate relationship between genetic diversity and relation to hybrid performance in pearl millet

Table 6. Genetic distance (GD) matrix among the eight maize inbred lines based on SSR analysis.

	B103	B104	B105	B106	B108	B109	B112	B113	B114	B115	B122	B123	B124	B125	B126	B134	B137	B142	R203	R208	R220	R247	R248	R249
B103	1	0.399	0.295	0.358	0.35	0.359	0.424	0.317	0.383	0.338	0.417	0.376	0.334	0.385	0.363	0.409	0.328	0.431	0.402	0.338	0.413	0.39	0.429	0.413
B104	0.399	1	0.479	0.242	0.304	0.305	0.40	0.302	0.307	0.32	0.402	0.381	0.319	0.55	0.548	0.594	0.552	0.454	0.508	0.444	0.477	0.454	0.493	0.448
B105	0.295	0.479	1	0.498	0.49	0.498	0.504	0.427	0.402	0.445	0.527	0.488	0.443	0.474	0.472	0.519	0.438	0.54	0.511	0.448	0.522	0.5	0.539	0.520
B106	0.358	0.242	0.498	1	0.293	0.384	0.446	0.291	0.358	0.309	0.391	0.35	0.308	0.338	0.338	0.583	0.541	0.483	0.497	0.433	0.488	0.443	0.454	0.438
B108	0.35	0.304	0.49	0.293	1	0.375	0.441	0.282	0.348	0.3	0.382	0.342	0.299	0.33	0.308	0.575	0.532	0.434	0.488	0.425	0.457	0.434	0.443	0.43
B109	0.359	0.305	0.498	0.384	0.375	1	0.211	0.343	0.408	0.361	0.443	0.402	0.399	0.39	0.388	0.435	0.384	0.455	0.497	0.434	0.465	0.443	0.413	0.403
B112	0.424	0.40	0.504	0.446	0.441	0.311	1	0.408	0.473	0.426	0.508	0.487	0.425	0.455	0.453	0.5	0.418	0.531	0.582	0.499	0.531	0.509	0.485	0.498
B113	0.317	0.383	0.427	0.291	0.282	0.343	0.408	1	0.194	0.229	0.265	0.27	0.228	0.258	0.259	0.503	0.299	0.492	0.455	0.202	0.424	0.402	0.413	0.367
B114	0.383	0.307	0.402	0.358	0.348	0.408	0.473	0.194	1	0.264	0.31	0.315	0.293	0.324	0.322	0.588	0.385	0.487	0.521	0.457	0.49	0.487	0.479	0.492
B115	0.338	0.32	0.445	0.309	0.3	0.361	0.426	0.229	0.264	1	0.329	0.266	0.228	0.257	0.255	0.181	0.317	0.42	0.473	0.41	0.442	0.42	0.431	0.415
B122	0.417	0.402	0.527	0.391	0.382	0.443	0.508	0.265	0.33	0.326	1	0.37	0.327	0.358	0.358	0.403	0.399	0.502	0.555	0.492	0.524	0.502	0.513	0.467
B123	0.376	0.381	0.498	0.35	0.342	0.402	0.487	0.27	0.325	0.268	0.37	1	0.225	0.192	0.252	0.543	0.559	0.481	0.515	0.451	0.483	0.481	0.472	0.495
B124	0.334	0.319	0.443	0.308	0.299	0.359	0.426	0.228	0.263	0.228	0.327	0.223	1	0.212	0.184	0.3	0.316	0.418	0.472	0.499	0.441	0.418	0.43	0.414
B125	0.385	0.35	0.474	0.338	0.33	0.36	0.495	0.299	0.324	0.297	0.358	0.352	0.212	1	0.241	0.531	0.347	0.449	0.503	0.439	0.473	0.449	0.491	0.444
B126	0.363	0.349	0.472	0.339	0.329	0.388	0.453	0.258	0.322	0.251	0.358	0.212	0.184	0.241	1	0.529	0.345	0.447	0.501	0.437	0.47	0.447	0.493	0.492
B134	0.409	0.394	0.519	0.393	0.375	0.425	0.5	0.303	0.388	0.391	0.403	0.343	0.3	0.331	0.329	1	0.392	0.494	0.548	0.484	0.517	0.494	0.505	0.489
B137	0.328	0.332	0.435	0.341	0.332	0.354	0.419	0.288	0.303	0.317	0.388	0.355	0.316	0.347	0.345	0.392	1	0.294	0.487	0.465	0.435	0.413	0.424	0.425
B142	0.431	0.451	0.54	0.412	0.434	0.458	0.521	0.402	0.487	0.42	0.502	0.481	0.418	0.449	0.447	0.494	0.294	1	0.589	0.506	0.538	0.515	0.527	0.51
R203	0.402	0.508	0.511	0.487	0.488	0.487	0.582	0.455	0.521	0.473	0.595	0.515	0.472	0.503	0.501	0.548	0.487	0.589	1	0.552	0.551	0.528	0.587	0.551
R208	0.338	0.444	0.448	0.433	0.425	0.434	0.498	0.382	0.457	0.41	0.482	0.451	0.409	0.439	0.437	0.484	0.493	0.505	0.552	1	0.485	0.485	0.504	0.498
R220	0.413	0.477	0.522	0.488	0.487	0.488	0.521	0.424	0.46	0.462	0.524	0.483	0.441	0.472	0.47	0.517	0.435	0.538	0.551	0.488	1	0.394	0.538	0.52
R247	0.39	0.454	0.5	0.443	0.434	0.443	0.506	0.402	0.487	0.42	0.502	0.481	0.418	0.448	0.447	0.494	0.413	0.515	0.528	0.485	0.394	1	0.514	0.485
R248	0.429	0.481	0.539	0.454	0.449	0.413	0.465	0.413	0.478	0.421	0.513	0.472	0.43	0.461	0.459	0.505	0.424	0.527	0.587	0.584	0.535	0.514	1	0.328
R249	0.413	0.449	0.523	0.438	0.43	0.403	0.498	0.387	0.482	0.418	0.487	0.456	0.414	0.444	0.442	0.499	0.498	0.51	0.551	0.488	0.52	0.498	0.328	1

Conclusion

This study demonstrated significant diversity among F_1 hybrids for all variables. Additive and non-additive gene effects contribute to the genetic regulation of all traits, with additive gene action predominating for the majority of traits. The selection of prospective hybrids ought to consider both general combining ability (GCA) and specific combining ability (SCA) effects. The inbred PMB 125 exhibited substantial and favourable GCA effects for head weight per plot, grain yield, single panicle weight, single panicle grain weight, and fodder yield per plot, signifying its potential to augment both grain and biomass productivity. PMB 133 and PMB 142 shown favourable general combining ability (GCA) effects for several traits, with PMB 142 presenting a large negative GCA effect for days to flowering, hence proving advantageous for the development of early-maturing cultivars. PMB 106 exhibited considerable GCA effects on productive tillers and yield-associated characteristics. Among the testers, PMR 247 emerged as a strong general combiner for robust yield characters.

The SCA study revealed several cross combinations exhibiting substantial and pronounced SCA impacts across multiple traits. Significant combinations comprised PMB115 x PMR203, PMB113 x PMR247, PMB106 x PMR230, and PMB122 x PMR203/PMB122 x PMR249, demonstrating enhanced performance in yield and biomass-related characteristics. High-yielding hybrids, including PMB137 x PMR248, PMB105 x PMR230, PMB123 x PMR247, and PMB142 x PMR249, exhibited significant hybrid vigour and commercial viability. The predicted genetic diversity based on SSR markers in this study could not be utilised to forecast the specific combining ability effects. The present investigation revealed a substantial correlation between genetic distance (GD) of parental inbred lines and the performance of F_1 hybrids across several important parameters. A robust association was discovered for plant height ($r = 0.77$), signifying that GD is a dependable predictor of plant stature in hybrids. Significant associations were identified for single panicle grain weight ($r = 0.61$) and grain yield ($r = 0.47$), indicating the efficacy of GD in forecasting critical agronomic characteristics. While SSR concluded that GD was ineffective in forecasting hybrid performance and SCA impacts, it proved proficient in categorising the inbred lines based on their origin, indicating the efficacy of SSR markers for diversification and clustering analysis. The top lines PMB 125, PMB 133, and PMB 142, in conjunction with tester PMR 247, exhibited significant GCA impacts, improving essential yield characteristics. High-yielding hybrids such as PMB115 x PMR203, PMB113 x PMR247, and PMB137 x PMR248 exhibited substantial SCA effects and strong vigour, underscoring their potential to improve grain and biomass production. Multi-location studies are crucial for validating performance across various environments for commercial utilization.

Acknowledgements

We want to thank VIT Bhopal and Rallis India Limited for providing the lab space and other research facilities

References

- Akinwale, R.; Badu-Apraku, B.; Fakorede, M.; Vroh-Bi, I. Heterotic grouping of tropical early-maturing maize inbred lines based on combining ability in Striga-infested and Striga-free environments and the use of SSR markers for genotyping. *Field Crop. Res.* 2014, *156*, 48–62.
- Badu-Apraku, B.; Oyekunle, M.; Fakorede, M.A.B.; Vroh, I.; Akinwale, R.O.; Aderounmu, M. Combining ability, heterotic patterns and genetic diversity of extra-early yellow inbreds under contrasting environments. *Euphytica* 2013, *192*, 413–433. [[CrossRef](#)]
- Bernardo, R. Relationship between single-cross performance and molecular marker heterozygosity. *Theor. Appl. Genet.* 1992, *83*, 628–634. [[CrossRef](#)] [[PubMed](#)]
- Betran, F.; Beck, D.; Bänziger, M.; Edmeades, G. Genetic analysis of inbred and hybrid grain yield under stress and nonstress environments in tropical maize. *Crop Sci.* 2003, *43*, 807–817. [[CrossRef](#)]
- Carena, M.J.; Hallauer, A.R.; Filho, J.M. Quantitative Genetics in Maize Breeding; Springer: New York, NY, USA, 2010
- Dangariya, C.J., Chotalia, J.M., Savaliya, J.J., Davda, B.K. and Pansuriya, A.G. 2009. Combining ability analysis in pearl millet (*Pennisetum glaucum* (L.) R. Br.). *Agric. Sci. Digest*, 29 (4): 287-290.
- Dhliwayo, T.; Pixley, K.; Menkir, A.; Warburton, M. Combining ability, genetic distances, and heterosis among elite CIMMYT and IITA tropical maize inbred lines. *Crop. Sci.* 2009, *49*, 1201–1210. [[CrossRef](#)]

Doyle, J. DNA Protocols for plants. In *Molecular Techniques in Taxonomy*; Springer Science and Business Media LLC: New York, NY, USA, 1991; pp. 283–293. Griffing, B. Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* 1956, 9, 463–493. [[CrossRef](#)]

Ejigu, Y.G.; Tongoona, P.B.; Ifie, B.E. General and specific combining ability studies of selected tropical white maize inbred lines for yield and yield related traits. *Int. J. Agric. Res. Innov. Technol.* 2017, 7, 381–396.

FAOSTAT.(2021).<https://www.fao.org/faostat/en/#data/QCL/visualize> (Assessed on 30th August 2023)

Gao BSB, Gangashetty PI, Mohammed R, Ango IK, Dzidzienyo DK, Tongoona P., Govindaraj M. Combining ability studies of grain Fe and Zn contents of pearl millet (*Pennisetum glaucum* L.) in West Africa. *Front Plant Sci.* 2023;13:1027279.

Gavali, K., Kute, N.S., Pawar, V.Y. and Patil, H.T. 2018. Combining ability analysis and gene action studies in pearl millet [*Pennisetum glaucum* (L.) R Br.]. *Ele. J. Plant Breeding*, 9 (3): 908-915

Geleta LF, Labuschagne MT, Viljoen CD (2004) Relationship between heterosis and genetic distance based on morphological traits and AFLP markers in pepper. *Plant Breed* 123:467–473.

Govindraj, M. 2011. Genetics of grain iron and zinc concentration in pearl millet (*Pennisetum glaucum* (L.) R. Br.). Ph. D. Thesis, TNAU, Coimbatore.

Gupta SK, Nepolean TV, Chinna GS, Rai KN, Hash CT, Das RR, Rathore A (2018) Phenotypic and molecular diversity-based prediction of heterosis in pearl millet (*Pennisetum glaucum* L. (R.) Br.). *Crop J* 6:271–281

Jaccard, P. Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaud. Sci. Nat.* 1908, 44, 223–270

- Kamara, M.M.; El-Degwy, I.S.; Koyama, H. Estimation combining ability of some maize inbred lines using line x tester mating design under two nitrogen levels. *Aust. J. Crop Sci.* 2014, 8, 1336
- Karvar SH, Pawar VY, Patil HT. Heterosis and Combining Ability in Pearl Millet (*Pennisetum glaucum* (L.) R. Br.). *Electronic J Plant Breed.* 2017;8(4):1197-1215.
- Khandagale, S.G., Sharma, V., Lone, R.P., Khandagale, V.G. and Kumara, R.V. 2014. Combining ability analysis and gene action in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Ele. J. Plant Breeding*, 5 (3): 445-450.
- Mageto, E.K.; Makumbi, D.; Njoroge, K.; Nyankanga, R. Genetic analysis of early-maturing maize (*Zea mays* L.) inbred lines under stress and nonstress conditions. *J. Crop. Improv.* 2017, 31, 560–588. [[CrossRef](#)]
- Makumbi, D.; Betrán, J.F.; Bänziger, M.; Ribaut, J.M. Combining ability, heterosis and genetic diversity in tropical maize (*Zea mays* L.) under stress and non-stress conditions. *Euphytica* 2011, 180, 143–162. [[CrossRef](#)]
- Menkir, A.; Melake-Berhan, A.; The, C.; Ingelbrecht, I.; Adepoju, A. Grouping of tropical mid-altitude maize inbred lines on the basis of yield data and molecular markers. *Theor. Appl. Genet.* 2004, 108, 1582–1590. [[CrossRef](#)] [[PubMed](#)]
- Obeng-Bio, E.; Badu-Apraku, B.; Ifie, B.E.; Danquah, A.; Blay, E.T.; Annor, B. Genetic analysis of grain yield and agronomic traits of early provitamin A quality protein maize inbred lines in contrasting environments. *J. Agric. Sci.* 2019, 157, 413–433. [[CrossRef](#)]
- Oppong, A.; Bedoya, C.A.; Ewool, M.B.; Asante, M.D.; Thomson, R.N.; Adu-Dapaah, H.; Lamptey, J.N.; Ofori, K.; Offei, S.K.; Warburton, M.L. Bulk genetic characterization of Ghanaian maize landraces using microsatellite markers. *Maydica* 2014, 59, 1–8.
- Oyekunle, M.; Badu-Apraku, B.; Hearne, S.; Franco, J. Genetic diversity of tropical early-maturing maize inbreds and their performance in hybrid combinations under

drought and optimum growing conditions. *Field Crop. Res.* 2015, 170, 55–65. [[CrossRef](#)]

Parentoni, S.; Magalhães, J.; Pacheco, C.; Santos, M.; Abadie, T.; Gama, E.; Guimarães, P.; Meirelles, W.; Lopes, M.; Vasconcelos, M.; et al. Heterotic groups based on yield-specific combining ability data and phylogenetic relationship determined by RAPD markers for 28 tropical maize open pollinated varieties. *Euphytica* 2001, 121, 197–208. [[CrossRef](#)]

Rai, K.N., Govindaraj, M. and Rao, A.S. 2012. Genetic enhancement of grain iron and zinc content in pearl millet. *Quality Assurance and Safety of Crop and Foods*, 4 (3): 119-125.

Ramya AR, Ahamed ML, Satyavathi CT, Rathore A, Katiyar P, Raj AGB, Kumar S, Gupta R, Mahendrakar MD, Yadav RS, Srivastava RK (2018) Towards Defning Heterotic Gene Pools in Pearl Millet [*Pennisetum glaucum* (L.) R. Br.]. *Front Plant Sci* 8:1–11.

Reif, J.C.; Gumpert, F.M.; Fischer, S.; Melchinger, A.E. Impact of interpopulation divergence on additive and dominance variance in hybrid populations. *Genetics* 2007, 176, 1931–1934. [[CrossRef](#)]

Riday H, Brummer EC, Campbell TA, Luth D, Cazcarro PM (2003) Comparisons of genetic and morphological distance with heterosis between *Medicago sativa* subsp. *sativa* and subsp. *falcata*. *Euphytica*

Shinde, G.C. (2011). Genetic studies and molecular analysis for forage yield in pearl millet (*Pennisetum glaucum* (L.) R. Br.). PhD Thesis (unpublished). MPKV, Rahuri.

Singh S, Gupta SK, Thudi M, Das RR, Vemula A, Garg V, Varshney RK, Rathore A, Pahuja SK, Yadav DV (2018) Genetic diversity patterns and heterosis prediction based on SSRs and SNPs in hybrid parents of pearl millet. *Crop Sci* 58(6):2379–2390

Singh, P. Genetic distance, heterosis and combining ability studies in maize for predicting F₁ hybrid performance. *Sabao J. Breed. Genet.* 2015, 47, 21–28.

Sprague, G.F.; Tatum, L.A. General vs. specific combining ability in single crosses of corn1. *Agron. J.* 1942, 34, 923–932. [[CrossRef](#)]

Sserumaga, J.P.; Ji, H.; Njoroge, K.; Muthomi, J.; Chemining'wa, G.; Si-myung, L.; Kim, H.; Asea, G.; Makumbi, D. Molecular characterization of tropical maize inbred lines using microsatellite DNA markers. *Maydica* 2014, 59, 267–274.

Statista, 2022. <https://www.statista.com/statistics/1286469/india-production-of-bajra/>

Teklewold A, Becker HC (2006) Comparison of phenotypic and molecular distances to predict heterosis and F₁ performance in Ethiopian mustard (*Brassica carinata* A. Braun). *Theor Appl Genet* 112:725–759.

Wei, T.; Simko, V.; Levy, M.; Xie, Y.; Jin, Y.; Zemla, J. Corrplot: Visualization of a Correlation Matrix. Available online: <https://github.com/taiyun/corrplot> (accessed on 15 December 2019). Botstein, D.; White, R.L.; Skolnick, M.; Davis, R.W. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 1980, 32, 314–331.

Zeid M, Schon CC, Link W (2003) Genetic diversity in recent elite faba bean lines using AFLP markers. *Theor Appl Genet* 107:1304–1314

Zhang, Y.; Fan, X.; Yao, W.; Piepho, H.P.; Kang, M.S. Diallel analysis of four maize traits and a modified heterosis hypothesis. *Crop. Sci.* 2016, 56, 1115–1126. [[CrossRef](#)]