

RESEARCH PAPER

Heterosis studies for yield and yield components using biofortified restorers on A₁ cms lines in pearl millet [*Pennisetum glaucum* (L.) R.]

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Abstract: In present investigation Line x Tester analysis was carried out to estimate heterosis (heterobeltiosis, average and standard heterosis) for identification of superior hybrids. Crosses attempted using 25 biofortified testers and two A₁ cytoplasm based male sterile lines during summer 2022-23. The resultant Fifty hybrids along with two male sterile lines, 25 testers and three checks viz., VPMH 7 (Local check), VPMH 14 (Local check) and Kaveri Super Boss (commercial private check) were planted at Regional Agricultural Research Station, Vijayapura during *kharif*, 2023 and evaluated for yield and yield components. Seven hybrids exhibited significant positive standard heterosis over check VPMH 7, one hybrid displayed substantial positive heterosis over the local check VPMH 14 and commercial private check Kaveri Super Boss for grain yield. In addition, hybrids ICMA 98222 x VJP Fe & Zn-9 and ICMA 98222 x VJP Fe & Zn-25 were found to be superior for dry fodder yield over check VPMH 7 which was found best check for dry fodder yield

Key words: A₁ cytoplasm, Biofortified restorers, Heterosis, Line x Tester

Introduction

Pearl millet (*Pennisetum glaucum*) is an important cereal crop primarily cultivated in arid and semi-arid regions around the world. This diploid species has a chromosome count of 2n=14 and belongs to the Poaceae (Gramineae) family. In India, pearl millet holds a prominent position as a vital source of dietary energy for impoverished farmers and consumers. It is also highly valued as a forage crop due to its lower hydrocyanic acid content compared to sorghum. The grain of pearl millet contains approximately 8.5 to 15% protein, 5.03 to 6.0% fat, 1.05 to 1.7% crude fiber and 65 to 70% carbohydrates. When used as a food crop, pearl millet grain boasts the highest calorie content per 100 grams, primarily derived from carbohydrates, fats and protein. Additionally, the grains are rich in two essential micronutrients viz., iron (18 to 135 ppm) and zinc (22 to 92 ppm) (Rai *et al.*, 2012).

Pearl millet ranks fourth in India in terms of area and production behind wheat, rice and maize. With an average productivity of 1430 kg/ha, pearl millet is grown over an area of around 6.84 mha in India and producing 9.78 mt. Rajasthan, Maharashtra, Uttar Pradesh, Gujarat and Haryana are the states that cultivate the most pearl millet accounting for 90 per cent of the production share of the country. In India, pearl millet is primarily grown in the rainy (*Kharif*) season (June/July). Additionally, it is grown during the summer months (February to May) in certain regions of Gujarat, Rajasthan and Uttar Pradesh as well as on a smaller scale in Maharashtra and Gujarat during the post-rainy (*Rabi*) season (November - February). In Karnataka, pearl millet is cultivated in an area of 1.48 lakh hectares with annual production of 1.71 lakh tonnes, with an average productivity of 1158 kg/ha (Anon., 2022).

Heterosis breeding is the foremost breeding method for improvement in cross pollinated crops. Heterosis studies also

help in rejecting a large number of crosses in F₁ generation itself and selecting only those having high yield potential. The identification of parental combinations that provide high heterosis for grain yield is the most important factor in hybrid development (Zhao *et al.*, 2015). Heterosis breeding provides an opportunity for utilization of available variability and generates new variability that is important for development of climate resilient hybrid varieties. With this view, the present work was undertaken to investigate the heterobeltiosis and standard heterosis for quantifying the extent of heterosis for grain yield and its component characters in pearl millet.

Material and methods

Experimental material was developed through crosses between 2 lines (Male sterile lines) viz., ICMA 98222 and ICMA 94555 and 25 testers (Restorer lines) viz., VJP Fe and Zn-1, VJP Fe and Zn-2, VJP Fe and Zn-3, VJP Fe and Zn-4, VJP Fe and Zn-5, VJP Fe and Zn-6, VJP Fe and Zn-7, VJP Fe and Zn-8, VJP Fe and Zn-9, VJP Fe and Zn-10, VJP Fe and Zn-11, VJP Fe and Zn-12, VJP Fe and Zn-13, VJP Fe and Zn-14, VJP Fe and Zn-15, VJP Fe and Zn-16, VJP Fe and Zn-17, VJP Fe and Zn-18, VJP Fe and Zn-19, VJP Fe and Zn-20, VJP Fe and Zn-21, VJP Fe and Zn-22, VJP Fe and Zn-23, VJP Fe and Zn-24 and VJP Fe and Zn-25 in Line x Tester design during summer 2022-23. Subsequently, the resulting 50 F₁ crosses along with parents (2 lines and 25 testers) and local checks VPMH 7, VPMH 14 and commercial check Kaveri Super Boss were evaluated at Regional Agricultural Research Station, Vijayapura during *kharif*, 2023 in a Randomized Block Design (RBD) with three replications. Each genotype was accommodated in 2 rows of 3 meter row length with a row spacing of 45 cm and plant to plant spacing of 15 cm.

From each entry/replication, five random, competitive plants were tagged and numbered in the middle of the row to observe

yield and other quantitative characters. Mean of the plants was computed and taken for analysis. The following observations were recorded: *viz.*, days to 50 per cent flowering, plant height (cm), SPAD chlorophyll meter readings at flowering and maturity stage, number of productive tillers per plant, panicle length (cm), panicle girth (cm), grain yield (kg ha⁻¹), dry fodder yield (kg ha⁻¹) and thousand seed weight (g).

Results and discussion

Analysis of variance (Table 1) revealed highly significant differences among parents and F₁ crosses for all 10 characters indicating existence of considerable genetic variability in the study material. However, it was discovered that the variation due to parents *vs.* crosses was significant for all the characters except panicle girth. For all the traits, parents showed significant variation. Likewise, lines recorded significant variation for six traits *viz.*, Days to fifty per cent flowering, plant height, number of productive tillers per plant, panicle length, grain yield and dry fodder yield whereas testers showed significant variation for all the traits. Variation due to Line × Tester revealed significant variation for days to 50 per cent flowering, plant height, number of productive tillers per plant, SPAD chlorophyll meter readings at flowering, SPAD chlorophyll meter readings at maturity, panicle length, panicle girth, dry fodder yield and thousand seed weight. Terms “positive” and “negative” heterosis indicated the trait value is increased or decreased as compared to the mean of the parents or to the better parent or checks VPMH 7, VPMH 14 and Kaveri Super Boss. Relative heterosis has less or no significance for plant breeders, so only heterobeltiosis and economic heterosis have been discussed. Development of high yielding genotypes despite that earliness (negative heterosis for number of days to 50% flowering) is also a major breeding objective for pearl millet breeding.

In pearl millet, earliness is desirable, therefore hybrids possessing negative heterotic effects were considered to be superior for days to 50% flowering. In this study, there were twenty-eight hybrids that displayed notable and substantial negative mid-parent heterosis. Additionally, thirty six crosses exhibited significant negative heterobeltiosis. Furthermore, only one hybrid showed significance in a negative direction when compared to the local check VPMH 7 and four hybrids exhibited negative significance in comparison to the local check VPMH 14. Moreover, all fifty crosses demonstrated significant negative heterosis when compared to the private check Kaveri Super Boss (Table 2). When considering specific cross combinations ICMA 94555 X VJP Fe & Zn-13 exhibited rapid flowering in just 42 days. From the results it can be concluded that hybrids flowered three to four days earlier than the parents indicating earliness is dominant. To summarize, the overall average heterosis for the time it took to reach 50 per cent flowering was -5.09 per cent as represented in Table 3 Similar findings were reported by Athoni *et al.*, 2021 and Yadav *et al.*, 2022.

Tallness is desirable in pearl millet, the hybrids possessing positive heterotic effects were therefore considered to be superior. Twenty nine hybrids exhibited significant positive relative heterosis and three hybrids demonstrated

Table 1. Analysis of variance (Mean sum of squares) for hybrids and parents with respect to morpho-phenological characters in pearl millet

Source	Df	Days to 50 per cent flowering	Plant height (cm)	Number of productive tillers per plant	SPAD chlorophyll meter readings at flowering	SPAD chlorophyll meter readings at maturity	Panicle length (cm)	Panicle girth (cm)	Grain yield (kg ha ⁻¹)	Dry fodder yield (kg ha ⁻¹)	Thousand seed weight
Replication	2	7.56	115.19	0.08	40.07	27.95	1.46	0.08	44309.64	7584930.33	1.96
Treatments	76	26.26**	795.22**	0.55**	50.70**	25.48**	7.49**	0.07**	1109687.60**	11761028.19**	5.42**
Parents	26	31.27**	1476.92**	0.46**	30.62*	23.42**	8.33**	1.72*	184568.61*	13249385.63**	3.16**
Parents <i>vs.</i> Cross	1	316.64**	880.93**	13.14**	363.16**	85.02**	156.14**	0.04	49899800.29**	31527038.85**	31.85**
Crosses/ Hybrids	49	17.67**	431.75**	0.35**	54.98**	25.36**	4.00**	0.08**	604850.47**	10567899.54**	6.07**
Line	1	80.67**	12357.88**	1.13**	18.73	2.04	6.41*	0.08	524995.18*	27687239.77**	0.09
Tester	24	31.10**	398.96**	0.40**	29.09*	22.87**	5.40**	0.07*	167695.82*	10929381.27**	2.88**
Line × Tester	1	10.08**	16467.09**	0.05**	79.21*	58.00*	80.67**	0.04	249089.06	54491636.31**	12.91**
Tester Error	152	1.12	71.09	0.08	17.18	9.50	1.32	0.04	102594.25	2012748.27	1.24
Total	230	9.48	310.75	0.24	28.46	14.94	3.36	0.05	434866.10	5282372.79	2.62

Table 2. Number of hybrids showing significant level of heterosis with respective direction and their ranges for morpho-phenological characters in pearl millet

Character	Mid parent heterosis			Heterobeltiosis					
	Positive (No.s)	Negative (No.s)	Range (%)	Positive (No.s)	Negative (No.s)	Range (%)			
Days to 50 per cent flowering	5	28	-16.07 to 18.92	2	36	-17.31 to 18.46			
Plant height (cm)	29	2	-20.05 to 50.47	3	18	-21.99 to 20.31			
No. of productive tillers per plant	24	1	-20.00 to 50.77	10	10	-28.57 to 48.48			
SPAD chlorophyll meter readings at flowering	18	1	-10.97 to 25.34	12	2	-13.67 to 21.73			
SPAD chlorophyll meter readings at maturity	16	0	-9.43 to 33.30	9	4	-15.45 to 32.75			
Panicle length (cm)	46	0	1.70 to 35.52	21	1	-9.68 to 22.52			
Panicle girth (cm)	4	1	-10.30 to 15.65	3	1	-11.90 to 16.90			
Grain yield (kg ha ⁻¹)	46	0	-1.09 to 104.47	38	0	-6.06 to 95.29			
Dry fodder yield (kg ha ⁻¹)	28	1	-26.88 to 128.17	13	10	-51.43 to 86			
Thousand seed weight (g)	25	1	-18.30 to 43.11	12	4	-22.79 to 42.23			
Character	Heterosis over VPMH 7			Heterosis over VPMH 14			Commercial heterosis over Kaveri Super Boss		
	Positive (No.s)	Negative (No.s)	Range (%)	Positive (No.s)	Negative (No.s)	Range (%)	Positive (No.s)	Negative (No.s)	Range (%)
Days to 50 per cent flowering	22	1	-4.55 to 16.67	5	25	-11.27 to 8.45	0	50	-24.10 to -7.23
Plant height (cm)	33	0	-8.33 to 31.08	2	8	-20.22 to 14.08	0	34	-26.42 to 5.22
No. of productive tillers per plant	8	3	-23.08 to 33.33	18	2	-18.92 to 40.54	18	2	-18.92 to 40.54
SPAD chlorophyll meter readings at flowering	2	4	-14.56 to 12.44	27	0	-3.12 to 27.50	19	0	-6.23 to 23.41
SPAD chlorophyll meter readings at maturity	4	0	-8.47 to 21.85	20	0	-1.82 to 30.70	2	3	-14.34 to 14.03
Panicle length (cm)	1	4	-14.01 to 8.89	0	7	-16.03 to 6.32	0	12	-16.52 to 5.70
Panicle girth (cm)	3	0	-11.54 to 19.23	2	1	-15.85 to 13.41	2	1	-15.85 to 13.41
Grain yield (kg ha ⁻¹)	7	3	-35.07 to 33.68	1	21	-42.72 to 17.94	1	19	-41.94 to 19.55
Dry fodder yield (kg ha ⁻¹)	2	18	-52.78 to 29.17	0	33	-58.02 to 14.81	0	42	-63.04 to 1.09
Thousand seed weight (g)	4	6	-28.40 to 18.84	19	1	-20.44 to 32.05	0	14	-32.98 to 11.24

heterobeltiosis. When assessing heterosis in comparison to reference checks, thirty three crosses showed considerable favorable heterosis against local check VPMH 7 and two crosses displayed similar substantial heterosis compared to local check VPMH 14. However, none of the crosses demonstrated superiority over the private check Kaveri Super Boss. In the case of the hybrids, the combination of ICMA 98222 x VJP Fe and Zn-10 produced the tallest plant height with a maximum of 198 cm. The average heterosis for plant height was recorded as -2.35 per cent as indicated in Table 3. Athoni *et al.*, 2021 and Yadav *et al.*, 2022 obtained similar results.

The hybrids ICMA 94555 X VJP Fe and Zn-16 (3.5) exhibited the greatest number of productive tillers per plant. Out of the fifty hybrids examined in this study, twenty four hybrids showed remarkable positive relative heterosis while three hybrids displayed heterobeltiosis. Furthermore, eight hybrids exhibited this effect in comparison to the local check VPMH 7. In addition, eighteen hybrids demonstrated significant favorable heterosis concerning the local check VPMH 14 and the same number of hybrids exhibited such heterosis when compared to the private check Kaveri Super Boss, as represented in Table 2. The average heterosis for the number of productive tillers per plant was recorded at 22.40 per cent as shown in Table 3. These outcomes

are in line with previous results of Athoni *et al.*, 2021; Suryawanshi *et al.*, 2021 and Yadav *et al.*, 2022.

The hybrid ICMA 94555 X VJP Fe and Zn-10 demonstrated the highest panicle length measuring 24.1 cm. The study revealed that a total of forty six hybrids showed noteworthy positive relative heterosis while twenty one hybrids displayed significant heterobeltiosis. Furthermore, one cross demonstrated significant positive heterosis when compared to the local check VPMH 7. However, none of the hybrids were identified as significant when compared to the local check, VPMH 14 and Kaveri Super Boss. Overall, the average heterosis for panicle length was recorded as 8.49 per cent (Table 3). The obtained results are similar to the results noticed by Krishnan *et al.*, 2019; Athoni *et al.*, 2021 and Suryawanshi *et al.*, 2021.

Among the hybrids ICMA 98222 X VJP Fe and Zn-15 and ICMA 98222 X VJP Fe and Zn-25 exhibited the greatest panicle girth measuring 3.1 cm. Four hybrids displayed favorable relative heterosis and three demonstrated heterobeltiosis. In comparison to the local check VPMH 7, three hybrids exhibited both significance and a positive effect. Furthermore, two crosses indicated significant positive effects when compared to the local check VPMH 14 and two crosses similarly showed significance in the desired direction compared to the Kaveri

Table 3. Range and mean performance of parents and their F₁s and average heterosis for morpho-phenological characters in pearl millet

Characters	Female parents			Male parents			Parental mean	Hybrids			Average Heterosis (%)
	Range		Mean	Range		Mean		Range		Mean	
	Min	Max		Min	Max			Min	Max		
Days to 50 per cent flowering	43	51	47	43	52	48	48	42	51	46	-5.09
Plant height (cm)	78	169	124	156	199	178	174	139	198	170	-2.35
No. of productive tillers per plant	2.2	3.1	2.7	1.7	3.1	2.2	2.2	2.1	3.5	2.7	22.40
SPAD chlorophyll meter readings at flowering	50.5	54.0	52.3	49.6	63.2	56.0	55.8	49.7	65.4	58.4	4.71
SPAD chlorophyll meter readings at maturity	36.1	37.3	36.7	35.5	46	39.9	39.7	36.0	48.0	41.0	3.20
Panicle length (cm)	15.7	17.8	16.8	18.1	23.2	20.6	20.3	19.9	24.0	22.0	8.49
Panicle girth (cm)	2.5	2.7	2.6	2.4	2.9	2.7	2.7	2.3	3.1	2.7	0.98
Grain yield (kg ha ⁻¹)	1647	2239	1943	1798	2567	2154	2139	2004	4126	3113	45.54
Dry fodder yield (kg ha ⁻¹)	3407	7704	5601	6370	12741	8687	8455	5037	13778	9229	9.16
Thousand seed weight (g)	11.3	11.5	11.4	11.4	14.8	12.9	12.8	9.8	16.2	13.6	6.07

Super Boss private check (Table 2). The overall mean heterosis for panicle girth was 0.98 *per cent* (Table 3). Many earlier researchers have also obtained the similar results (Krishnan *et al.*, 2019; Athoni *et al.*, 2021 and Suryawanshi *et al.*, 2021).

Among the fifty cross combinations, the hybrid ICMA 98222 × VJP Fe and Zn-25 (13778 kg/ha) achieved the most substantial dry fodder yield per hectare. Twenty eight hybrids displayed significant positive mid-parent heterosis and similarly thirteen hybrids surpassed the better parent heterosis measure. The two hybrids demonstrated superiority over the local check VPMH 7. Additionally, none of the crosses exhibited desired direction of heterosis over local check VPMH 14 and private check Kaveri Super Boss. The favorable heterotic effect identified in this study is likely attributed mainly to the diversity among the parental traits (Table 2). On average, the heterosis for dry fodder yield per hectare was found to be 9.16 *per cent* as indicated in Table 3. These observations are in line with the reports of previous workers *i.e.*, Athoni *et al.* (2021a), Suryawanshi *et al.* (2021) and Yadav *et al.* (2022).

Grain yield per hectare represents the crucial trait that holds the breeders attention as every breeding approach strives to enhance yield in the end. Out of the 60 cross combinations, the hybrid ICMA 98222 X VJP Fe and Zn-18 achieved the highest grain yield reaching a remarkable 4126 kg/ha. Forty six hybrids displayed noteworthy positive mid-parent heterosis while thirty eight hybrids surpassed the measurement of better parent heterosis. Seven hybrids demonstrated superiority over the local check VPMH 7. Additionally, one cross exhibited the desired direction of heterosis when compared to the VPMH 14 check and another cross did the same when compared to the Kaveri Super Boss check (Table 2). The average heterosis was calculated to be 45.54 *per cent* (Table 3). Many investigators have also reported similar outcomes (Krishnan *et al.*, 2019; Athoni *et al.*, 2021 and Suryawanshi *et al.*, 2021).

Burton (1951) documented heterosis in terms of grain yield per plant while Ahluwalia and Patnaik (1963) identified heterobeltiosis. They highlighted that factors affecting yield such as seed size, panicle girth and panicle length exhibit a multiplicative nature. Consequently, they concluded that even

a slight improvement in these yield related characteristics could lead to higher grain yield. These findings are in line with this observation. Therefore, it can be inferred that heterotic effects associated with yield determining traits like panicle length, panicle girth, the number of productive tillers per plant and thousand seed weight significantly contribute to the extent of heterosis.

The thousand seed weight of a genotype functions as a sign of the grain yield as it is a chief component impacting yield. Among the hybrids, the pairing ICMA 94555 X VJP Fe and Zn-13 secured the highest thousand seed weight measuring at 16.2 g. Twenty five crosses exhibited notable positive significant performance in relation to mid-parent heterosis whereas twelve hybrids displayed significant positive heterobeltiosis. Additionally, four crosses were significantly superior to VPMH 7 and nineteen crosses showed substantial superiority to VPMH 14. Meanwhile, no hybrids showed significant superiority over the private check Kaveri Super Boss. The mean heterosis for thousand seed weight was witnessed to be 6.07 *per cent* (Table 3). Similar results were obtained by Athoni *et al.*, 2021 and Suryawanshi *et al.*, 2021.

Conclusions

The current study on relative heterosis, heterobeltiosis and standard heterosis over three checks including local checks VPMH 7, VPMH 14 and commercial check Kaveri Super Boss, revealed that, crosses had a heterotic effect for all the characters studied. For most of the traits, significant positive heterosis over mid and better parent was observed. Only one cross (ICMA 98222 X VJP Fe and Zn-18) was concluded as superior hybrid which exhibited positive heterosis over the best check VPMH 14 for grain yield. In addition, hybrids ICMA 98222 x VJP Fe and Zn-9 and ICMA 98222 X VJP Fe and Zn-25 were found to be superior with respect to dry fodder yield over check VPMH 7 which was found best check for fodder yield. Hence, these hybrids could be evaluated on large scale for their potentiality. The magnitude of average heterosis for grain yield was 45.54 *per cent* which is due to positive average heterosis for yield contributing characters like number of productive tillers per plant, thousand seed weight, panicle girth and panicle length.

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