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Heterosis Analysis for Grain Yield and Its Attributing Traits in Pearl Millet [*Pennisetum glaucum* (L.) R. Br.)] Using Cytoplasmic Male Sterile Based Hybrids

Patel, R.N [a++*], Kumar, A [a++] and Pareek, B [a++]

[a] *School of Agriculture, Singhania University, Pacheri Bari, Jhunjhunu, Rajasthan, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A line × tester mating design involving five CMS lines and nine restorer lines of pearl millet [*Pennisetum glaucum* (L.) R. Br.] was used to generate 45 F_1 hybrids, which were evaluated along with their parents and a standard check hybrid (GHB 1129) for nine agronomic and yield-related traits. The hybrids showed substantial variation for most traits, and the analysis of variance indicated ample genetic variability and significant differences among both female and male parents. Marked heterotic responses were observed particularly for grain yield per plant, number of effective tillers per plant, earhead length, and harvest index, whereas days to 50% flowering, earhead girth, and test weight expressed comparatively lower to moderate levels of heterosis. Among the crosses,

[++] *Research Scholar;*
**Corresponding author: E-mail: roshanpatel145k@gmail.com;*

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ICMA 15666 × HBL-11 R consistently recorded the highest relative heterosis and maximum heterobeltiosis for grain yield per plant, followed by ICMA 15666 × ICMR 11666, indicating the strong yield-enhancing potential of ICMA 15666-based hybrids. Based on per se performance and standard heterosis, four hybrids—ICMA 15666 × HBL-11 R, 7A × ICMR 0911, ICMA 14222 × ICMR 17333, and ICMA 21999 × ICMR 08222—were identified as superior to GHB 1129 for grain yield per plant, with several of them also expressing desirable heterosis for earhead length, earhead girth, and test weight. The predominance of highly heterotic combinations, particularly those involving complementary CMS and restorer parents, highlights the effectiveness of the line × tester approach for identifying elite hybrid combinations and provides promising genetic material for developing high-yielding, heterosis-based pearl millet hybrids suited to dryland production environments.

Keywords: Pearl millet; LxT mating design; heterosis; earhead girth.

1. INTRODUCTION

Pearl millet (*Cenchrus americanus* (L.) Morrone; synonym *Pennisetum glaucum* (L.) R. Br.) is one of the most ancient and resilient cereal crops cultivated in arid and semi-arid regions of the world. Commonly known by various vernacular names such as Bajra in Hindi, Sajje in Kannada, Kambu in Tamil, Bajeer in Kumaoni, and Maiwa in Hausa, pearl millet represents a vital component of traditional farming systems across Africa and the Indian subcontinent. Archaeobotanical evidence suggests that its domestication dates back several thousand years, with Africa recognized as its primary center of origin and India as a major secondary center of diversification. Over centuries, it has played a crucial role in ensuring food and nutritional security in dryland regions characterized by erratic rainfall, poor soil fertility, and high temperatures. Among the small millets cultivated globally, pearl millet holds the foremost position in terms of area and production. In India, it ranks first among all millets, followed by sorghum (Sorghum bicolor) and finger millet (Eleusine coracana). It contributes significantly to the livelihoods of millions of smallholder and marginal farmers, particularly in states such as Rajasthan, Gujarat, Maharashtra, Haryana, and Uttar Pradesh, where it serves as both a staple food and an important fodder crop. On a global scale, the crop accounts for nearly 14 million tonnes of annual grain production, with major contributions from India, Niger, Nigeria, and Sudan.

Nutritionally, pearl millet is often referred to as a “nutricereal” due to its superior composition compared to major cereals like rice and wheat. It is a rich source of carbohydrates, proteins, and essential micronutrients such as iron, zinc, magnesium, and phosphorus. The grain also contains considerable amounts of dietary fiber, unsaturated fatty acids, and bioactive compounds, which contribute to improved digestive health, glycemic control, and cardiovascular well-being. Owing to its high nutritional value and gluten-free nature, pearl millet has been gaining renewed attention in the context of health-conscious diets and sustainable food systems. Despite its nutritional richness and adaptability to harsh environments, the consumption and cultivation of pearl millet have declined in many parts of the world. Changing dietary preferences, urbanization, and the policy bias towards major cereals have led to its marginalization. To reverse this trend and promote sustainable millet-based agriculture, India has taken several initiatives. The Government of India declared 2018 as the “National Year of Millets” to enhance public awareness, encourage research and development, and strengthen value-chain integration. This initiative was followed by a global recognition when the United Nations General Assembly, with the support of more than 70 member nations, declared 2023 as the “International Year of Millets”, a milestone that underscored the global importance of millets in achieving food security, climate resilience, and nutritional well-being.

Pearl millet’s adaptability to high temperatures, drought tolerance, and low input requirements make it a promising crop under changing climatic scenarios. It serves as an excellent model for studying resilience mechanisms in cereals, with ongoing research focusing on genetic improvement for yield, stress tolerance, and nutrient use efficiency. Moreover, the crop’s potential extends beyond traditional food uses—it is increasingly being explored for industrial applications such as bioethanol production, animal feed, and functional food products.

Therefore, understanding the agronomic, nutritional, and genetic aspects of pearl millet is essential for promoting its sustainable cultivation and utilization. With its dual role in ensuring food security and supporting climate-smart agriculture, pearl millet stands as a cornerstone crop for the dryland tropics and a key component of future global food systems. Pearl millet (*Cenchrus americanus* (L.) Morrone; synonym *Pennisetum glaucum* (L.) R. Br.) is the most drought-tolerant of all domesticated cereals (Govindaraj *et al.* , 2010). It thrives in the hottest and driest regions where most other cereals fail due to drought, high temperature, and poor soil fertility. Because of this remarkable adaptability, pearl millet serves as a principal staple food for more than 90 million people living in the Sahel region of Africa and the arid fringes of the Thar Desert in India (Gulia *et al.* , 2007). Beyond its role as a food grain, pearl millet is also grown as a forage and cover crop in certain parts of the United States. It produces high-quality and high-yielding summer grazing forage (Burton and Fortson, 1966) and performs well as a grain crop under drought-prone and nutrient-deficient soils due to its efficient water and nutrient utilization compared with crops such as sorghum and maize (Muchow, 1988; Maman *et al.*, 2006). In recent years, the increasing global demand for gluten-free food products and millet-based flours, particularly among African and Asian immigrant communities, has further enhanced the market potential of pearl millet grain in the United States (Gulia *et al.*, 2007).

Pearl millet is a highly cross-pollinated crop with an outcrossing rate exceeding 85%, showing a strong expression of heterosis for both grain and stover yield. In the 1950s, attempts were made to exploit this heterosis using the protogynous nature of flowering in the crop. The common practice during this period involved growing the parental lines together and allowing natural cross-pollination to occur. The resulting seed lots contained about 40% hybrid seed when the parental lines flowered synchronously. These chance hybrids yielded 10–15% higher than the existing local varieties; however, they did not gain widespread popularity because of their limited yield advantage over open-pollinated varieties (OPVs), narrow adaptability, and the absence of structured seed production programs. Breeding methods for pearl millet have mainly followed approaches designed for cross-pollinated crops. Its genetic improvement has primarily relied on conventional hybridization followed by selection for developing composites, synthetics, and hybrids. The introduction and use of cytoplasmic-genetic male sterility (CMS) systems significantly improved the efficiency of hybrid seed production and expanded opportunities for large-scale hybrid development. In addition, population improvement techniques and the integration of modern biotechnological approaches have provided new possibilities for genetic enhancement of this crop.

The effectiveness of hybrid breeding in improving productivity has been clearly demonstrated in several major crops such as maize, cotton, sorghum, castor, rice, tobacco, and pearl millet (Banga and Banga, 1998). Today, hybrid breeding, combined with molecular and genomic tools, continues to play a major role in boosting pearl millet yield potential, stress tolerance, and adaptability to harsh agro-climatic conditions.

2. MATERIALS AND METHODS

The present investigation was carried out at Ketanbhai Patel Farm, Ishanpur Mota village, Taluka & District Gandhinagar, Gujarat 23.2193956° N latitude and 72.7785897° E longitude. Soil at the experimental site was classified as sandy loam. The experimental material consisted of five CMS lines as female parent and Nine restorer lines as male parent (Table 1). The parents crossed in line × tester mating design during Kharif season of 2023 at Ketanbhai Patel Farm Village Ishanpur Mota, Ta & Dist. Gandhinagar, Gujarat. The replication wise mean values were used for statistical analysis.

A set of 60 genotypes comprising of fourteen parents and their 45 F_1 hybrids along with standard check GHB-1129 were sown in Randomized Block Design (RBD) with three replications, during *Summer* 2024. Each entry sown in 4 m length row with 45 cm × 15 cm spacing. The recommended agronomical practices and plant protection measures were adopted as per requirement.

Five plants were randomly selected and tagged from each net plot of parents and F1's in all the replications to record the periodical observations. Recording the various observations like, Days to 50 % flowering, Days to maturity, Plant height (cm), Number of effective tiller per plant, Earhead length (cm), Ear head girth (mm), Test weight (g), Grain yield per plant (g) and Seed setting (%).

Table 1. List of parental lines and their source that selected for present investigation

Sr. no.	Lines (Females)	Source	Sr. No.	Testers (Male)	Source
1	7 A	H.A.U., Hissar	1	HBL 11 R	H.A.U., Hissar
2	37 A	H.A.U., Hissar	2	AC 04/B R	H.A.U., Hissar
3	ICMA 14222	ICRISAT, Hyderabad	3	ICMR 09111	ICRISAT, Hyderabad
4	ICMA 15666	ICRISAT, Hyderabad	4	ICMR 09777	ICRISAT, Hyderabad
5	ICMA 21999	ICRISAT, Hyderabad	5	ICMR 11111	ICRISAT, Hyderabad
			6	ICMR 11666	ICRISAT, Hyderabad
			7	ICMR 17333	ICRISAT, Hyderabad
			8	ICMR 08222	ICRISAT, Hyderabad
			9	ICMR 12777	ICRISAT, Hyderabad

Heterosis calculations: Heterosis was estimated as percentage deviation using standard formulae:

Per cent heterosis over mid-parent (MP) (Relative heterosis) $= \frac{\overline{F_1} - \overline{MP}}{\overline{MP}} \times 100$

Per cent heterosis over better parent (BP) (Heterobeltiosis) $= \frac{\overline{F_1} - \overline{BP}}{\overline{BP}} \times 100$

Per cent heterosis over standard parent (GDM 4) (Standard heterosis) $= \frac{\overline{F_1} - \overline{SC}}{\overline{SC}} \times 100$

Where,

$\overline{F_1}$: Mean performance of F_1 hybrid
$\overline{MP}$: Mean performance of mid-parent
$\overline{BP}$: Mean performance of better parent
$\overline{SC}$: Mean performance of standard parent

Note: Clearly state in the manuscript whether the "better parent" is the higher or lower value for each trait (e.g., for days to 50% flowering lower is better if earliness is desirable).

The replication wise mean values of each entry for the various traits were analysed using Randomized Block Design (RBD) as suggested by Panse and Sukhatme (1985). The averaged mean values of the different traits was subjected to statistical analysis to test the significance of variation for the experiment conducted under the RBD field design as per model of Panse and Sukhatme (1985). Heterosis was estimated as per cent increase or decrease by the formulae used in estimation of heterosis over mid-parent (Briggle, 1963), better parent (Fonseca and Patterson, 1968) and heterosis over standard check (Meredith and Bridge, 1972).

3. RESULTS AND DISCUSSION

3.1 Heterosis

Heterosis, or hybrid vigour, has become one of the most valuable genetic approaches for enhancing crop yield and productivity. Identifying specific parental combinations that can generate superior heterotic effects in F_1 hybrids is a crucial step in the success of hybrid breeding programs. The commercial utilization of heterosis represents a major advancement in plant breeding, as it has contributed significantly to yield improvement in numerous crop species. Although the estimation of heterosis over the mid-parent value is of theoretical interest, its practical relevance is limited. In contrast, heterosis measured over the better parent provides a more realistic and meaningful assessment for plant breeders. The ultimate commercial worth of a hybrid largely depends on its superiority relative to the best existing variety or hybrid. Therefore, in the present investigation, heterosis was assessed in terms of both better-parent heterosis (heterobeltiosis) and standard heterosis to identify parental combinations exhibiting superior performance. These results aim to guide the development of promising transgressive segregants and assist in characterizing parental lines with potential utility in future pearl millet improvement programs.

For an effective heterosis breeding strategy, it is essential that hybrids display significant and desirable heterotic effects. In estimating heterobeltiosis for days to flowering, days to maturity, and plant height, the parent with the lower mean value was considered the better parent. Analysis of mid-parent heterosis for these traits revealed that, among the 45 hybrids evaluated, seven expressed significant heterosis in the desirable (negative) direction. Only four hybrids showed significant negative heterobeltiosis, while none exhibited significant negative standard heterosis for these traits. These observations align with the earlier reports of Patel *et al.* (2016) and Karvar *et al.* (2017). In the case of heterobeltiosis for plant height, none of the 45 hybrids exhibited significant negative heterosis over the better parent. However, 11 crosses showed significant negative standard heterosis for this trait. These observations are in close agreement with the findings of Karvar *et al.* (2017) and Yadav *et al.* (2022).

Table 2. Estimates of heterosis in percentage in F_1 hybrid over mid parent, better parent and standard check GHB 1129 for days to 50% flowering and days to maturity in pearl millet

Sr. no.	Hybrids	Days to 50% flowering			Days to maturity		
		MP	BP	SC	MP	BP	SC
1	7 A x HBL-11 R	-0.33	4.11*	5.56**	-0.21	2.61*	3.51**
2	7 A x AC 04/13 R	-0.65	3.40	5.56**	-0.42	2.16	3.51**
3	7 A x ICMR 0911	1.96	6.12**	8.34**	1.27	3.90**	5.27**
4	7 A x ICMR 09777	-6.29**	-6.29**	3.48	-4.12**	-4.12**	2.20
5	7 A x ICMR 11111	-4.55**	-1.34	2.09	-2.94**	-0.86	1.32
6	7 A x ICMR 11666	-0.65	2.68	6.25**	-0.42	1.72	3.95**
7	7 A x ICMR 17333	-3.85*	-1.96	4.17*	-2.50*	-1.27	2.64*
8	7 A x ICMR 08222	-0.33	4.11*	5.56**	-0.21	2.61*	3.51**
9	7 A x ICMR 12777	-0.65	2.68	6.25**	-0.42	1.72	3.95**
10	37 A x HBL-11 R	2.39	2.74	4.17*	1.52	1.74	2.64*
11	37 A x AC 04/13 R	-2.04	-2.04	0.00	-1.30	-1.30	0.00
12	37 A x ICMR 0911	0.00	0.00	2.09	0.00	0.00	1.32
13	37 A x ICMR 09777	-6.54**	-2.72	-0.70	-4.22**	-1.73	-0.44
14	37 A x ICMR 11111	-1.35	-0.68	1.39	-0.86	-0.43	0.88
15	37 A x ICMR 11666	7.43**	8.16**	10.42**	4.74**	5.19**	6.58**
16	37 A x ICMR 17333	-2.67	-0.68	1.39	-1.71	-0.43	0.88
17	37 A x ICMR 08222	-2.39	-2.05	-0.70	-1.52	-1.30	-0.44
18	37 A x ICMR 12777	-1.35	-0.68	1.39	-0.86	-0.43	0.88
19	ICMA 14222 x HBL-11 R	-0.33	2.05	3.48	-0.21	1.30	2.20
20	ICMA 14222 x AC 04/13 R	-2.67	-0.68	1.39	-1.71	-0.43	0.88
21	ICMA 14222 x ICMR 0911	-2.00	0.00	2.09	-1.28	0.00	1.32
22	ICMA 14222 x ICMR 09777	-6.41**	-4.58**	1.39	-4.17**	-2.95**	0.88
23	ICMA 14222 x ICMR 11111	-2.65	-1.34	2.09	-1.70	-0.86	1.32
24	ICMA 14222 x ICMR 11666	-1.32	0.00	3.48	-0.85	0.00	2.20
25	ICMA 14222 x ICMR 17333	-4.58**	-4.58**	1.39	-2.95**	-2.95**	0.88
26	ICMA 14222 x ICMR 08222	-0.33	2.05	3.48	-0.21	1.30	2.20
27	ICMA 14222 x ICMR 12777	-6.62**	-5.37**	-2.09	-4.26**	-3.43**	-1.32
28	ICMA 15666 x HBL-11 R	-0.34	0.68	2.09	-0.22	0.43	1.32
29	ICMA 15666 x AC 04/13 R	1.35	2.04	4.17*	0.86	1.30	2.64*
30	ICMA 15666 x ICMR 0911	-3.38	-2.72	-0.70	-2.16	-1.73	-0.44
31	ICMA 15666 x ICMR 09777	-3.25	0.00	3.48	-2.10	0.00	2.20
32	ICMA 15666 x ICMR 11111	8.72**	8.72**	12.50**	5.58**	5.58**	7.90**
33	ICMA 15666 x ICMR 11666	6.71**	6.71**	10.42**	4.29**	4.29**	6.58**
34	ICMA 15666 x ICMR 17333	-3.31	-2.01	1.39	-2.13	-1.29	0.88
35	ICMA 15666 x ICMR 08222	9.83**	10.96**	12.50**	6.26**	6.96**	7.90**
36	ICMA 15666 x ICMR 12777	6.71**	6.71**	10.42**	4.29**	4.29**	6.58**
37	ICMA 21999 x HBL-11 R	10.58**	10.96**	12.50**	6.72**	6.96**	7.90**

Sr. no.	Hybrids	Days to 50% flowering			Days to maturity		
		MP	BP	SC	MP	BP	SC
38	ICMA 21999 x AC 04/13 R	14.29**	14.29**	16.67**	9.09**	9.09**	10.53**
39	ICMA 21999 x ICMR 0911	10.20**	10.20**	12.50**	6.49**	6.49**	7.90**
40	ICMA 21999 x ICMR 09777	-1.96	2.04	4.17*	-1.27	1.30	2.64*
41	ICMA 21999 x ICMR 11111	-1.35	-0.68	1.39	-0.86	-0.43	0.88
42	ICMA 21999 x ICMR 11666	2.70	3.40	5.56**	1.72	2.16	3.51**
43	ICMA 21999 x ICMR 17333	4.00*	6.12**	8.34**	2.56*	3.90**	5.27**
44	ICMA 21999 x ICMR 08222	4.44*	4.79**	6.25**	2.82*	3.04**	3.95**
45	ICMA 21999 x ICMR 12777	1.35	2.04	4.17*	0.86	1.30	2.64*
Range		-6.62 to 14.29	-10.06 to 14.29	-2.90 to 16.67	-4.26 to 9.09	-6.58 to 9.09	-1.32 to 7.90
Significant Heterosis		17	17	22	17	17	22
No. of +ve significant		10	13	22	10	13	22
No. of -ve significant		7	4	0	7	4	0

Table 3. Estimates of heterosis in percentage in F1 hybrid over mid parent, better parent and standard check GHB 1129 for Plant height and Number of effective tillers in pearl millet

Sr. no.	Hybrids	Plant height (cm)			Number of effective tiller per plant		
		MP	BP	SC	MP	BP	SC
1	7 A x HBL-11 R	40.29**	62.04**	-6.62**	-4.72	-23.42**	31.38*
2	7 A x AC 04/13 R	92.13**	90.62**	9.87**	-36.42**	-54.82**	11.84
3	7 A x ICMR 0911	-12.35**	37.12**	-20.98**	-24.44*	-34.11**	-7.71
4	7 A x ICMR 09777	16.89**	82.89**	5.41**	28.30	6.25	10.75
5	7 A x ICMR 11111	31.66**	85.15**	6.71**	-47.64**	-59.78**	-21.83
6	7 A x ICMR 11666	33.42**	67.74**	-3.33*	-11.90	-22.92	-19.66
7	7 A x ICMR 17333	45.34**	85.50**	6.91**	111.11**	58.33**	65.04**
8	7 A x ICMR 08222	17.17**	83.70**	5.87**	14.46	-1.04	3.15
9	7 A x ICMR 12777	-11.79**	38.38**	-20.25**	121.43**	61.46**	68.30**
10	37 A x HBL-11 R	41.33**	67.49**	-7.72**	-36.74**	-37.34**	7.50
11	37 A x AC 04/13 R	67.10**	69.58**	-6.58**	13.32*	-4.82	135.62**
12	37 A x ICMR 0911	-8.37**	47.84**	-18.55**	33.80**	22.58**	106.30**
13	37 A x ICMR 09777	10.17**	77.78**	-2.05	40.37**	-1.29	66.13**
14	37 A x ICMR 11111	21.37**	75.74**	-3.18*	-20.96**	-26.26**	43.33**
15	37 A x ICMR 11666	51.48**	95.72**	7.84**	-31.28**	-49.68**	-15.31
16	37 A x ICMR 17333	43.83**	88.71**	3.98**	-0.49	-34.84**	9.67
17	37 A x ICMR 08222	19.19**	92.71**	6.18**	-41.33**	-57.42**	-28.34*
18	37 A x ICMR 12777	25.33**	102.80**	11.74**	58.79**	1.94	71.56**
19	ICMA 14222 x HBL-11 R	42.63**	40.63**	9.25**	59.17**	20.89**	107.39**
20	ICMA 14222 x AC 04/13 R	48.74**	28.68**	-0.05	-40.65**	-59.65**	-0.11
21	ICMA 14222 x ICMR 0911	25.44**	61.78**	25.68**	-38.39**	-49.61**	-29.43*
22	ICMA 14222 x ICMR 09777	6.41**	37.25**	6.62**	164.83**	134.15**	108.47**

Sr. no.	Hybrids	Plant height (cm)			Number of effective tiller per plant		
		MP	BP	SC	MP	BP	SC
23	ICMA 14222 x ICMR 11111	10.58**	29.64**	0.71	-26.44**	-46.37**	4.24
24	ICMA 14222 x ICMR 11666	9.21**	15.96**	-9.93**	-27.27	-31.71*	-39.20**
25	ICMA 14222 x ICMR 17333	38.09**	48.58**	15.42**	55.38**	23.17	9.67
26	ICMA 14222 x ICMR 08222	6.28**	37.34**	6.69**	142.11**	124.39**	99.79**
27	ICMA 14222 x ICMR 12777	14.93**	48.61**	15.45**	47.62*	13.41	0.98
28	ICMA 15666 x HBL-11 R	66.15**	74.63**	19.64**	-37.80**	-58.86**	-29.43*
29	ICMA 15666 x AC 04/13 R	86.16**	70.15**	16.57**	-70.61**	-82.02**	-55.49**
30	ICMA 15666 x ICMR 0911	28.78**	79.72**	23.12**	-27.78*	-49.61**	-29.43*
31	ICMA 15666 x ICMR 09777	17.69**	64.26**	12.54**	73.68**	57.14**	7.50
32	ICMA 15666 x ICMR 11111	22.83**	55.05**	6.23**	-48.70**	-67.04**	-35.94**
33	ICMA 15666 x ICMR 11666	57.30**	78.85**	22.53**	31.71	12.50	-12.06
34	ICMA 15666 x ICMR 17333	46.89**	69.38**	16.04**	106.06**	100.00**	10.75
35	ICMA 15666 x ICMR 08222	10.89**	55.05**	6.23**	68.60**	45.71**	10.75
36	ICMA 15666 x ICMR 12777	29.80**	81.62**	24.42**	118.95**	103.92**	12.93
37	ICMA 21999 x HBL-11 R	38.86**	40.76**	3.45*	-19.33	-39.24**	4.24
38	ICMA 21999 x AC 04/13 R	70.83**	51.35**	11.23**	-59.74**	-72.81**	-32.69**
39	ICMA 21999 x ICMR 0911	1.92	36.04**	-0.02	-2.39	-20.93*	10.75
40	ICMA 21999 x ICMR 09777	0.00	33.49**	-1.90	-45.45**	-51.25**	-57.66**
41	ICMA 21999 x ICMR 11111	8.80**	31.72**	-3.20*	-39.77**	-56.42**	-15.31
42	ICMA 21999 x ICMR 11666	32.69**	45.14**	6.67**	26.32	20.00	4.24
43	ICMA 21999 x ICMR 17333	4.34*	15.70**	-14.98**	26.56	1.25	-12.06
44	ICMA 21999 x ICMR 08222	1.68	35.98**	-0.07	25.33	17.50	2.07
45	ICMA 21999 x ICMR 12777	1.68	36.07**	0.01	6.45	-17.50	-28.34*
Range		-12.35 to 92.13	15.70 to 102.80	-20.98 to 25.68	-70.61 to 164.83	-82.02 to 134.15	-57.66 to 135.62
Significant Heterosis		41	45	38	32	32	19
No. of +ve significant		38	45	27	15	10	10
No. of -ve significant		3	0	11	17	22	9

Table 4. Estimates of heterosis in percentage in F1 hybrid over mid parent, better parent and standard check GHB 1129 for Ear head length and Ear head girth in pearl millet

Sr. No.	Hybrids	Ear head length (cm)			Ear head girth (mm)		
		MP	BP	SC	MP	BP	SC
1	7 A x HBL-11 R	1.33	-16.95**	31.42**	-4.09	-14.89**	6.27
2	7 A x AC 04/13 R	14.54**	-22.76**	22.23**	-3.42	-24.03**	-5.15
3	7 A x ICMR 0911	16.94**	6.54**	68.59**	21.24**	17.67**	56.12**
4	7 A x ICMR 09777	-43.01**	-44.85**	-6.71	-16.49**	-24.28**	-5.46
5	7 A x ICMR 11111	-26.62**	-35.59**	1.92	-9.72*	-16.90**	3.77
6	7 A x ICMR 11666	9.77**	-18.40**	29.12**	7.26	-11.26*	10.80*
7	7 A x ICMR 17333	16.06**	-5.93*	48.86**	-4.15	-4.51	19.24**
8	7 A x ICMR 08222	8.01**	5.33*	66.67**	-16.51**	-26.06**	19.71**
9	7 A x ICMR 12777	5.56*	3.51	63.80**	-10.63*	-12.14**	9.71
10	37 A x HBL-11 R	17.13**	6.82	8.05*	24.47**	17.93**	14.09*
11	37 A x AC 04/13 R	0.14	-16.78**	-30.66**	34.78**	23.10**	6.58
12	37 A x ICMR 0911	19.39**	-2.06	27.40**	3.92	-14.13**	13.93*
13	37 A x ICMR 09777	-4.70	-28.88**	20.31**	22.43**	13.38*	15.18**
14	37 A x ICMR 11111	6.14	-9.94**	7.67*	29.85**	18.45**	24.40**
15	37 A x ICMR 11666	69.18**	62.76**	35.64**	34.63**	30.87**	13.30*
16	37 A x ICMR 17333	31.01**	21.05**	18.97**	14.33**	-2.90	20.34**
17	37 A x ICMR 08222	32.95**	3.31	55.37**	10.69*	-15.06**	37.53**
18	37 A x ICMR 12777	-1.87	-24.06**	15.52**	36.05**	16.84**	40.96**
19	ICMA 14222 x HBL-11 R	0.16	-12.78**	18.97**	10.02*	-6.64	29.56**
20	ICMA 14222 x AC 04/13 R	1.40	-28.79**	-2.88	0.74	-23.65**	5.96
21	ICMA 14222 x ICMR 0911	3.52	1.12	37.94**	-5.12	-7.21	28.78**
22	ICMA 14222 x ICMR 09777	-14.48**	-22.76**	30.66**	6.63	-7.66	28.15**
23	ICMA 14222 x ICMR 11111	23.65**	16.01**	58.24**	28.46**	12.84**	56.59**
24	ICMA 14222 x ICMR 11666	12.39**	-12.08**	19.93**	34.66**	6.98	48.47**
25	ICMA 14222 x ICMR 17333	16.08**	-0.14	36.21**	12.91**	6.87	48.31**
26	ICMA 14222 x ICMR 08222	-11.96**	-16.05**	26.25**	-19.65**	-25.39**	20.81**
27	ICMA 14222 x ICMR 12777	-10.62**	-15.24**	28.93**	-12.05**	-17.79**	14.09*
28	ICMA 15666 x HBL-11 R	44.33**	32.08**	60.92**	33.73**	22.64**	42.21**
29	ICMA 15666 x AC 04/13 R	54.98**	12.58**	37.17**	63.50**	32.21**	53.31**
30	ICMA 15666 x ICMR 0911	23.19**	19.29**	55.18**	22.94**	15.19**	52.84**
31	ICMA 15666 x ICMR 09777	-12.57**	-24.80**	27.21**	13.22**	6.20	23.15**
32	ICMA 15666 x ICMR 11111	8.57**	7.55*	31.04**	34.65**	28.30**	48.78**
33	ICMA 15666 x ICMR 11666	68.79**	37.74**	67.82**	63.00**	38.95**	61.12**
34	ICMA 15666 x ICMR 17333	36.99**	23.74**	50.77**	25.99**	21.94**	51.12**

Sr. No.	Hybrids	Ear head length (cm)			Ear head girth (mm)		
		MP	BP	SC	MP	BP	SC
35	ICMA 15666 x ICMR 08222	5.14	-4.84	43.11**	16.09**	-0.39	61.28**
36	ICMA 15666 x ICMR 12777	25.73**	13.22**	72.23**	7.93	5.83	27.68**
37	ICMA 21999 x HBL-11 R	-2.91	-15.35**	15.14**	8.52	-6.13	24.40**
38	ICMA 21999 x AC 04/13 R	55.71**	9.44**	48.86**	26.95**	-2.24	29.56**
39	ICMA 21999 x ICMR 0911	21.96**	19.30**	62.27**	14.20**	14.13**	51.43**
40	ICMA 21999 x ICMR 09777	0.94	-8.95**	54.03**	20.69**	6.60	41.28**
41	ICMA 21999 x ICMR 11111	-5.10	-10.85**	21.27**	21.71**	9.08*	44.56**
42	ICMA 21999 x ICMR 11666	40.47**	10.00**	49.62**	43.25**	15.80**	53.47**
43	ICMA 21999 x ICMR 17333	-0.90	-14.65**	16.10**	11.03**	7.43	42.37**
44	ICMA 21999 x ICMR 08222	-3.81	-8.41**	37.74**	-10.08**	-18.24**	32.37**
45	ICMA 21999 x ICMR 12777	3.86	-1.64	49.62**	23.46**	17.92**	56.28**
Range		-43.01 to 69.18	-44.85 to 62.76	-30.66 to 72.23	-19.65 to 63.50	-26.06 to 38.95	-5.46 to 61.28
Significant Heterosis		30	35	42	35	31	38
No. of +ve significant		24	14	41	28	18	38
No. of -ve significant		6	21	1	7	13	0

Table 5. Estimates of heterosis in percentage in F1 hybrid over mid parent, better parent and standard check GHB 1129 for Test Weight (gm) and Grain yield/plant(g) in pearl millet

Sr. no.	Hybrids	Test weight (gm)			Grain yield/plant (g)		
		MP	BP	SC	MP	BP	SC
1	7 A x HBL-11 R	13.04**	-11.94**	-0.33	140.36**	120.72**	118.19
2	7 A x AC 04/13 R	34.73**	30.06**	-17.86**	43.27**	40.92**	20.48**
3	7 A x ICMR 0911	26.87**	2.49	5.15	192.40**	146.98**	196.31**
4	7 A x ICMR 09777	-21.72**	-33.33**	-40.13**	43.66**	41.83**	20.36**
5	7 A x ICMR 11111	61.02**	57.46**	4.06	12.64**	-9.88**	24.17**
6	7 A x ICMR 11666	11.34**	0.47	-21.14**	8.53*	2.77	-15.01**
7	7 A x ICMR 17333	-11.16**	-30.87**	-21.51**	43.43**	38.59**	22.90**
8	7 A x ICMR 08222	21.93**	-1.77	1.50	127.95**	123.89**	91.98**
9	7 A x ICMR 12777	-26.52**	-44.35**	-31.73**	101.53**	83.88**	84.35**
10	37 A x HBL-11 R	-5.65	-21.94**	-11.65**	82.36**	70.27**	68.32**
11	37 A x AC 04/13 R	4.40	-6.40	-30.64**	173.55**	173.15**	134.22**
12	37 A x ICMR 0911	-20.66**	-31.67**	-29.91**	137.35**	103.50**	144.15**
13	37 A x ICMR 09777	15.81**	5.69	-5.08	106.86**	105.79**	76.46**
14	37 A x ICMR 11111	40.63**	33.00**	-1.43	-24.19**	-38.50**	-15.27**
15	37 A x ICMR 11666	41.15**	37.21**	7.71**	133.78**	117.66**	86.64**
16	37 A x ICMR 17333	16.34**	-3.86	9.17**	164.77**	160.40**	130.92
17	37 A x ICMR 08222	-34.57**	-43.82**	-41.95**	88.43**	88.43**	61.58**
18	37 A x ICMR 12777	-1.67	-21.13**	-3.25	32.97**	23.35**	23.66**
19	ICMA 14222 x HBL-11 R	-2.25	-22.90**	-12.75**	152.32**	127.80**	125.19**
20	ICMA 14222 x AC 04/13 R	52.35**	44.69**	-5.44	81.20**	75.00**	49.61**
21	ICMA 14222 x ICMR 0911	26.96**	3.91	6.61*	134.42**	95.02**	133.97**

Sr. no.	Hybrids	Test weight (gm)			Grain yield/plant (g)		
		MP	BP	SC	MP	BP	SC
22	ICMA 14222 x ICMR 09777	54.82**	33.74**	20.12**	27.76**	23.84**	5.09
23	ICMA 14222 x ICMR 11111	46.67**	45.86**	-3.62	122.35**	75.44**	141.73**
24	ICMA 14222 x ICMR 11666	84.26**	68.84**	32.54**	206.55**	195.53**	135.69**
25	ICMA 14222 x ICMR 17333	34.29**	5.79*	20.12**	224.26**	207.75**	172.90**
26	ICMA 14222 x ICMR 08222	0.00	-18.37**	-15.67**	150.46**	141.54**	107.12**
27	ICMA 14222 x ICMR 12777	30.49**	0.00	22.68**	142.86**	117.89**	118.45**
28	ICMA 15666 x HBL-11 R	-20.44**	-29.68**	-20.41**	235.95**	213.90**	210.30**
29	ICMA 15666 x AC 04/13 R	7.27	-10.08**	-21.87**	16.56**	16.30**	-0.13
30	ICMA 15666 x ICMR 0911	-16.38**	-22.78**	-20.78**	127.32**	95.02**	133.97**
31	ICMA 15666 x ICMR 09777	4.13	2.44	-8.00**	-8.64*	-9.19*	-22.01**
32	ICMA 15666 x ICMR 11111	29.36**	13.87**	-1.06	23.21**	0.00	37.79**
33	ICMA 15666 x ICMR 11666	25.39**	19.33**	3.69	229.62**	206.67**	163.36**
34	ICMA 15666 x ICMR 17333	-7.10*	-18.01**	-6.91*	103.50**	100.29**	77.61**
35	ICMA 15666 x ICMR 08222	-5.95*	-13.43****	-10.56**	16.53**	16.44**	0.00
36	ICMA 15666 x ICMR 12777	6.97**	-8.63**	12.09**	48.74**	38.07**	38.42**
37	ICMA 21999 x HBL-11 R	-36.78**	-48.71**	-41.95**	-21.67**	-25.35**	-26.20**
38	ICMA 21999 x AC 04/13 R	37.85**	26.42**	-10.92**	57.85**	54.26**	38.17**
39	ICMA 21999 x ICMR 0911	20.25**	1.42	4.06	91.38**	67.13**	100.51**
40	ICMA 21999 x ICMR 09777	-17.08**	-26.02**	-33.56**	171.77**	164.63**	137.02**
41	ICMA 21999 x ICMR 11111	1.60	-1.55	-30.64**	22.22**	0.83	38.93**
42	ICMA 21999 x ICMR 11666	36.76**	29.77**	1.87	46.30**	33.52**	19.59**
43	ICMA 21999 x ICMR 17333	-11.51**	-28.30**	-18.59**	34.90**	34.23**	20.23**
44	ICMA 21999 x ICMR 08222	-21.85**	-34.28**	-32.10**	210.30**	203.69**	172.01**
45	ICMA 21999 x ICMR 12777	22.50**	-3.57	18.30**	66.22**	57.36**	57.76**
Range		-36.78 to 84.26	-48.71 to 68.84	-41.95 to 32.54	-24.19 to 235.95	-38.50 to 213.90	-26.20 to 210.30
Significant Heterosis		37	23	32	45	41	40
No. of +ve significant		24	13	9	42	38	36
No. of -ve significant		13	20	23	3	3	4

Table 6. Estimates of heterosis in percentage in F1 hybrid over mid parent, better parent and standard check GHB 1129 for Seed Set (%) in pearl millet

Sr. no.	Hybrids	Seed set (%)		
		MP	BP	SC
1	7 A x HBL-11 R	-5.73*	-8.60**	-9.58**
2	7 A x AC 04/13 R	3.58	2.23	-2.49
3	7 A x ICMR 0911	-1.65	-5.61*	-4.61
4	7 A x ICMR 09777	1.86	-0.72	-2.84
5	7 A x ICMR 11111	2.55	-1.75	-0.36
6	7 A x ICMR 11666	5.34*	1.78	1.42
7	7 A x ICMR 17333	4.55	3.76	-2.13
8	7 A x ICMR 08222	-1.92	-2.67	-9.58**
9	7 A x ICMR 12777	8.29**	7.25**	-0.36
10	37 A x HBL-11 R	1.09	-0.36	-1.42
11	37 A x AC 04/13 R	-1.48	-1.85	-5.68*
12	37 A x ICMR 0911	1.44	-1.05	0.00
13	37 A x ICMR 09777	-2.74	-3.62	-5.68*
14	37 A x ICMR 11111	-5.21*	-7.69**	-6.39*
15	37 A x ICMR 11666	3.26	1.42	1.07
16	37 A x ICMR 17333	0.19	-0.74	-4.61
17	37 A x ICMR 08222	2.46	0.00	-3.91
18	37 A x ICMR 12777	-3.79	-6.27*	-9.93**
19	ICMA 14222 x HBL-11 R	3.26	2.15	1.07
20	ICMA 14222 x AC 04/13 R	5.90*	5.13*	1.78

Sr. no.	Hybrids	Seed set (%)		
		MP	BP	SC
21	ICMA 14222 x ICMR 0911	-3.23	-5.26*	-4.26
22	ICMA 14222 x ICMR 09777	-5.28*	-5.80*	-7.81**
23	ICMA 14222 x ICMR 11111	-10.55**	-12.59**	-11.35**
24	ICMA 14222 x ICMR 11666	-1.44	-2.85	-3.20
25	ICMA 14222 x ICMR 17333	6.12*	4.76	1.42
26	ICMA 14222 x ICMR 08222	5.46*	2.56	-0.71
27	ICMA 14222 x ICMR 12777	-1.89	-4.76	-7.81**
28	ICMA 15666 x HBL-11 R	2.63	-2.15	-3.20
29	ICMA 15666 x AC 04/13 R	-1.53	-4.46	-8.87**
30	ICMA 15666 x ICMR 0911	7.43**	1.40	2.49
31	ICMA 15666 x ICMR 09777	6.99**	2.54	0.36
32	ICMA 15666 x ICMR 11111	0.56	-5.24*	-3.91
33	ICMA 15666 x ICMR 11666	-3.00	-7.83**	-8.16**
34	ICMA 15666 x ICMR 17333	5.59**	3.01	-2.84
35	ICMA 15666 x ICMR 08222	8.02**	6.98*	-2.13
36	ICMA 15666 x ICMR 12777	-2.75	-3.50	-12.06**
37	ICMA 21999 x HBL-11 R	-0.89	-1.07	-1.78
38	ICMA 21999 x AC 04/13 R	-6.01*	-7.86**	-8.52**
39	ICMA 21999 x ICMR 0911	1.24	0.35	1.42
40	ICMA 21999 x ICMR 09777	-3.96	-4.64	-5.32*
41	ICMA 21999 x ICMR 11111	-6.36*	-7.34**	-6.03*
42	ICMA 21999 x ICMR 11666	-9.80**	-9.96**	-10.29**
43	ICMA 21999 x ICMR 17333	1.10	-1.43	-2.13
44	ICMA 21999 x ICMR 08222	5.20*	1.07	0.36
45	ICMA 21999 x ICMR 12777	1.68	-2.50	-3.20
Range		-10.55 to 8.29	-12.59 to 7.25	-12.60 to 2.49
Significant Heterosis		17	15	16
No. of +ve significant		10	3	0
No. of -ve significant		7	12	16

For number of effective tillers per plant, 15 hybrids exhibited significant positive heterosis over the mid-parent, 10 hybrids showed significant positive heterobeltiosis, and 10 hybrids demonstrated significant positive standard heterosis in the desirable direction. These results corroborate the findings of Karvar *et al.* (2017), Yadav *et al.* (2022), and Bhatiya *et al.* (2025). Regarding earhead length, out of 45 crosses, 24 hybrids recorded significant positive mid-parent heterosis, 14 hybrids showed significant positive heterobeltiosis, and 41 hybrids expressed significant positive standard heterosis in the desired direction. These findings are consistent with those of Karvar *et al.* (2017) and Bhatiya *et al.* (2025). For earhead girth, 28 hybrids exhibited significant positive mid-parent heterosis, 18 hybrids showed significant positive heterobeltiosis, and 38 hybrids displayed significant positive standard heterosis over the check hybrid GHB 1129. These results align with the observations reported by Karvar *et al.* (2017), Ingle *et al.* (2023), and Bhatiya *et al.* (2025). For test weight, 13 hybrids exhibited significant positive heterobeltiosis, while nine hybrids showed significant positive standard heterosis, corroborating the findings of Karvar *et al.* (2017) and Bhatiya *et al.* (2025). Concerning grain yield per plant, 42 hybrids expressed significant positive mid-parent heterosis, and 36 hybrids demonstrated significant positive standard heterosis. These results are in close agreement with the findings of Karvar *et al.* (2017), Yadav *et al.* (2022), Ingle *et al.* (2023), and Bhatiya *et al.* (2025). The results showed that phenotypic distance could be a better predictor of heterosis than molecular distance. The correlation between phenotypic distance and heterosis was not strong enough to permit the use of phenotypic diversity among parents as a major selection criterion for selection of parental lines displaying high levels of heterosis for grain and stover yield in pearl millet (Gupta *et al.*, 2018).

For seed setting, only 10 hybrids showed significant positive mid-parent heterosis, three hybrids exhibited significant positive heterobeltiosis, and seven hybrids expressed significant positive standard heterosis. These results substantiate the observations reported by Sumathi and Revathi (2017). Based on the heterotic effects, the hybrid ICMA 15666 × HBL-11 R exhibited significant positive heterosis (in the desirable direction) over the standard check for earhead length, earhead girth, and grain yield per plant. The second hybrid, 7A × ICMR 0911, demonstrated significant positive heterosis over the standard check for plant height, earhead length, earhead girth, and grain yield per plant. The third hybrid, ICMA 14222 × ICMR 17333, showed significant standard heterosis for earhead length, earhead girth, test weight, and grain yield per plant, while the fourth hybrid, ICMA 21999 × ICMR 08222, similarly exhibited significant standard heterosis for earhead length, earhead girth, test weight, and grain yield per plant.

4. SUMMARY AND CONCLUSION

The analysis of variance (ANOVA) for the experimental design indicated that the mean squares due to genotypes were significant for all the characters studied, confirming the presence of ample genetic variability within the experimental material. The comparison of parents versus hybrids showed significant differences for all traits except days to 50% flowering, days to maturity, number of effective tillers per plant, and seed setting, suggesting the occurrence of mean heterosis for these traits. Further partitioning of the parental genotypes revealed that the mean squares attributed to female parents were highly significant for all traits except days to 50% flowering, days to maturity, and seed setting, whereas male parents exhibited highly significant differences for all characters. This result indicates a considerable amount of genetic diversity among both female and male parental lines.

The magnitude of heterosis varied across the hybrids. A higher degree of heterosis was observed for grain yield per plant, number of effective tillers per plant, earhead length, and harvest index, whereas traits such as days to 50% flowering, earhead girth, and test weight exhibited low to moderate levels of heterosis. The cross ICMA 15666 × HBL-11 R recorded the highest relative heterosis for grain yield per plant, followed by ICMA 15666 × ICMR 11666. The hybrid ICMA 15666 × HBL-11 R also showed the maximum heterobeltiosis for grain yield per plant. These promising crosses exhibited high heterosis for several yield-attributing traits in the desirable direction, demonstrating their potential utility for commercial exploitation of heterosis.

The greatest standard heterosis values were obtained in the hybrids ICMA 15666 × HBL-11 R and 7A × ICMR 0911. Interestingly, a higher frequency of heterotic hybrids originated from low × high specific combining ability (SCA) parental combinations compared to high × high or low × low general combining ability (GCA) combinations for all traits evaluated. Based on the heterotic response, ICMA 15666 × HBL-11 R expressed significant positive heterosis over the standard check for earhead length, earhead girth, and grain yield per plant, while 7A × ICMR 0911 exhibited significant positive heterosis for plant height, earhead length, earhead girth, and grain yield per plant.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

I hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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