

ID RA1526

Exploitation of heterosis for enhanced biomass and nutritional superiority in forage pearl millet (*Pennisetum glaucum* L.)

Pramit P. Patel, Y. A. Viradiya, Dhruv Pankajkumar Joshi*, Kishor K. Varotariya, Dixita H. Padhiyar and Vasu R. Davra

Department of Genetics and Plant Breeding, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar

*Correspondence author email id: joshidhruvp@gmail.com

ORCID: <https://orcid.org/0009-0007-9054-9097>

Received: 01-07-2026

Accepted: 17-08-2026

ABSTRACT

By 2050, green fodder scarcity for livestock in India is estimated to reach 18.50%. With limited scope for horizontal land expansion, increasing forage productivity through genetic improvement is essential. Fodder pearl millet can meet this challenge due to its rapid growth, drought tolerance, high biomass accumulation, and absence of toxic hydrogen cyanide (HCN). Therefore, this study was conducted to identify highly vigorous elite F_1 hybrids by estimating standard heterosis and heterobeltiosis across 14 morphological, yield, phenological, and nutritional traits. A total of 54 genotypes, including 40 F_1 hybrids were developed *via* a Line $\times$ Tester mating design, and evaluated in *kharif* 2024 in Randomized Block Design. Analysis of variance showed highly significant genetic variability among the genotypes for all the traits. The hybrid 'AFB-66 $\times$ RBB-1' showed exceptional performance, recording the highest green fodder yield (175.67 g/plant) alongside a standard heterosis of 31.42%. Also, 'AFB-23 $\times$ GIANT BAJRA' and 'AFB-23 $\times$ BAIF' showed higher total biomass. 'AFB-67 $\times$ BAIF' was the early maturing hybrids (-19.15% standard heterosis), while 'AFB-23 $\times$ AFB-45' had higher dry matter accumulation (21.76% standard heterosis), which is showing superior combinations of qualitative and developmental traits. Improvements in leaf morphology with better palatability, were noticeable in crosses like 'AFB-25 $\times$ BAIF'. Regarding nutritional quality, 'AFB-21 $\times$ ICMO-1604' showed a 42.86% standard heterosis for crude protein, and 'AFB-66 $\times$ ICMO-1604' showed a 13.92% standard heterosis for iron content. Noticeably, HC-20 $\times$ ICMO-1604 showed both higher crude protein content (9.30%) and higher iron content (11.09 mg/100g) compared to check. The study concludes that targeted crossing of diverse lines can simultaneously enhance vegetative bulk, dry matter density, phenological adaptability, and nutritional quality. Also, this study identified specific elite crosses with immense potential for commercial release, and thus increasing forage production in the country. The analysis of heterosis of the presented hybrids will be helpful for further top cross analysis which is important for forage breeding.

Keywords : Forage pearl millet, Green fodder yield, Heterobeltiosis, Standard heterosis, Nutritional density, *Pennisetum glaucum*

INTRODUCTION

India has one of the world's biggest livestock populations, which makes the animal husbandry sector a cornerstone of national development and the rural economy. The global agricultural framework heavily depends on livestock production, which is 25.6% of India's agricultural GDP (Anonymous, 2024). However, achieving increased milk production and higher farmer income requires a strong focus on animal feed management, particularly on green fodder procurement. In recent situation, the sustainability of this sector is heavily affected by a noticeable decrease in animal feed. Only 4% of India's cultivable land is allocated to green fodder cultivation (Dhamodharan *et al.*, 2024), leading to estimation of an 18.4% deficit in green fodder by

2050. Because allocation of more land for fodder cultivation is unfeasible due to increasing competition for food grain production, the only way left is a paradigm shift toward increasing forage productivity per unit area through the adoption of scientific cultivation methods.

Pearl millet (*Pennisetum glaucum* L., $2n = 2X = 14$) emerges as a critical crop for narrowing this gap. As a fast-growing, short-duration C_4 cereal grass, it shows remarkable resistance to water scarcity and heat, allowing it to be grown successfully across various soil types. Its photosynthetic efficiency is higher than C_3 crops by fifty percent (Wang *et al.*, 2012), enabling rapid biomass accumulation even in drought-prone, arid, and semi-arid conditions. Furthermore, pearl millet is uniquely suited for forage utilization. It contains a high amount of dry matter

and crude protein, which makes it a prominent summer and monsoon fodder crop. It provides high digestibility for ruminants, exceptional tillering for multi-cut regeneration, and crucially, an absence of toxic hydrogen cyanide (HCN) (Bhattacharya and Mandal, 2003). This lack of HCN makes it completely safe for cattle at all growth stages, unlike forage sorghum.

To magnify these physiological advantages and enable farmers to increase their income through the production of superior fodder, modern plant breeding relies heavily on the exploitation of heterosis. As an outcrossing C_4 species, pearl millet displays substantial hybrid vigor for vegetative growth and biomass accumulation (K. Srivastava *et al.*, 2020). Apart from grain yield, systematic application of heterosis for optimizing vegetative biomass and green fodder quality remains an urgent priority, particularly because non-additive gene action strongly influences traits such as crude protein content and dry matter digestibility (Govintharaj *et al.*, 2021). Furthermore, while many experimental hybrids show superiority over their parental lines, assessing both better-parent heterosis and standard heterosis against commercial checks is mandatory to determine true agronomic viability (Choudhary, 2023). Therefore, the present investigation was carried out to rigorously estimate the magnitude of standard heterosis and heterobeltiosis in newly developed forage pearl millet hybrids.

MATERIAL AND METHODS

Plant material and Experimental design

The experimental material consisted of 54 forage pearl millet genotypes, which included eight lines used as females (HC-20, AFB-44, AFB-21, AFB-24, AFB-67, AFB-23, AFB-25, and AFB-66), five testers as males (BAIF, GIANT BAJRA, ICMO-1604, RBB-1, and AFB-45), their 40 single crosses, and one standard check variety (GAFF-4). The F_1 hybrids were developed utilizing a Line $\times$ Tester mating design during the summer 2024 at the Centre for Forage Research, S. D. Agricultural University, Sardarkrushinagar, Gujarat. To ensure controlled pollination, female and pollen parent ear heads were protected with butter paper bags before stigma emergence, and hand-pollination was carried out during morning hours (8:00 am to 11:30 am).

The evaluation of the complete set of genotypes was conducted during the *khari* 2024 season. The experiment was laid out in a Randomized Block Design (RBD) with three replications. Each genotype was planted in a single-row plot measuring 4.0 meters in length, maintaining a row-to-row spacing of 30 cm and a plant-to-plant spacing of 10 cm.

Data collection

Standard agronomical and plant protection protocols were

maintained throughout the crop cycle. Phenotypic observations were recorded on five randomly selected, competitive plants per plot in each replication for morphological and yield traits. The genotypes were evaluated for traits namely plant height (cm), number of tillers per plant, stem diameter (cm), leaves per plant, leaf length (cm), internode length (cm), leaf width (cm), leaf to stem ratio, green fodder yield per plant (g), and dry matter yield per plant (g) while, days to 50% flowering, dry matter content (%), crude protein content (*via* Kjeldahl method; AOAC, 2005), and iron content were assessed on a whole-plot basis. For iron content determination, dried samples were digested using a nitric acid-perchloric acid mixture and analyzed *via* an Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES; Model Optima 8000, PerkinElmer Inc., Waltham, MA, USA) following the protocol described by Wheal *et al.* (2011).

Statistical Analysis

The mean values of the observational data were subjected to an analysis of variance (ANOVA) based on the fixed-effect model described by Panse and Sukhatme (1985) to establish the significance of genotypic differences. To evaluate hybrid superiority, heterosis was calculated as the percentage increase or decrease of the F_1 hybrid over its corresponding better parent (heterobeltiosis) and the standard check (standard heterosis). Heterobeltiosis (HB) was estimated according to Fonseca and Patterson (1968). Standard heterosis (SH) was calculated following the methodology of Meredith and Bridge (1972).

RESULTS AND DISCUSSION

As per the Fig. 1, the phenotypic ranges of the 14 evaluated traits vary significantly in both absolute scale and dispersion for all the genotypes. Yield and quality traits like, green fodder yield per plant, dry matter yield per plant, and crude protein, showed the widest divergence between minimum and maximum values. This is the indication of a broad genetic base. Plant height showed upper logarithmic scale and leaf : stem ratio showed lower end. Conversely, the constrained ranges observed in days to flowering and leaf length suggest a lower degree of variability and higher stability within the evaluated population."

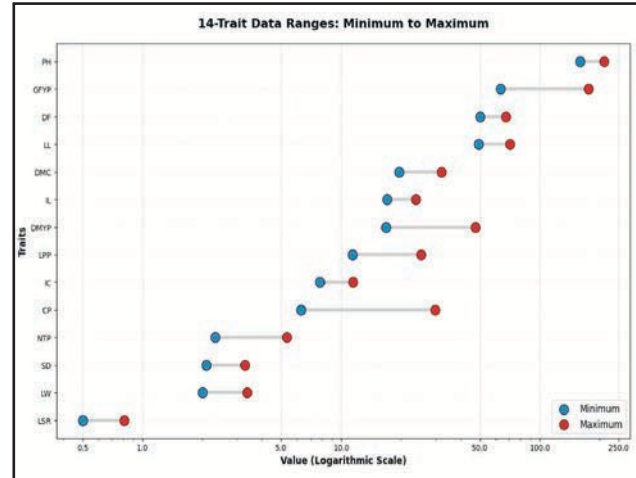
The analysis of variance (Table 1) confirmed highly significant differences among the genotypes, parents, and hybrids for all fourteen traits evaluated, indicating a vast amount of genetic variability within the experimental material. When comparing parents *versus* hybrids, the mean squares were significant for nearly all traits (except days to flowering, leaf width, dry matter content), which strongly points to the presence of substantial heterotic effects.

Summary of five top performing hybrids from forty crosses, in terms of desired standard heterosis; with their respective heterobeltiosis and mean data for 14 traits in forage

pearlmillet is shown in Table 2. In practical plant breeding, the assessment of heterosis in relation to the superior parent and/or a standard check variety is more applicable and has greater practical significance. Therefore, the primary objective of the heterosis analysis in this study was to identify the optimal combinations that yield a high level of heterobeltiosis and standard heterosis. This information will be helpful for generating improved transgressive segregants, as well as to characterize the parent varieties for their potential contributions to future breeding programs.

Biomass production is the ultimate goal in forage breeding. For green fodder yield per plant, the hybrid 'AFB-66 × RBB-1' demonstrated exceptional *per se* performance, recording the highest mean yield of 175.67 g. This was closely followed by 'AFB-23 × GIANT BAJRA' (167.00 g) and 'AFB-23 × BAIF' (160.67 g) (Table 2). Fig. 2 showing complete data of heterobeltiosis and standard heterosis for green fodder yield per plant for all 40 crosses, which is showing the range of heterotic responses beyond the top-performing crosses summarized in Table 2.

However, high *per se* yield performance must be supported by heterotic superiority. The cross 'AFB-66 × RBB-1' exhibited a massive heterobeltiosis of 105.85% and a highly significant standard heterosis of 31.42% over the check variety GAFB-4 for green fodder yield. Similarly, 'AFB-23 × GIANT BAJRA' recorded a 114.10% increase over its better parent and a 24.94% increase over the standard check for the same trait. Also, the trend is followed by 'AFB-23 × BAIF' for standard heterosis (20.20%).



DF-Days to flowering, PH-Plant height, NTP-Number of tillers per plant, SD-Stem diameter, LPP-Leaves per plant, L.L-Leaf length, IL-Internode length, L.W-Leaf width, LSR-Leaf: stem ratio, GFYP-Green fodder yield per plant, DMYP-Dry matter yield per plant, DMC-Dry matter content, CP-Crude Protein content, IC-Iron content

Figure 1: The absolute phenotypic amplitude across the 14 evaluated traits. The dumbbell plot illustrates the spread from the minimum to the maximum recorded values on a logarithmic scale for 54 genotypes

Table 1 : Analysis of variance for experimental design for different characters in forage pearl millet

Source of variation	Df	Days to flowering	Plant height	Number of tillers per plant	Stem diameter	Leaves per plant	Leaf length	Internode length
Replication	2	17.16	361.80	0.18	0.07	3.93	12.24	2.04
Genotypes	53	56.06**	616.60**	1.17**	0.20**	30.77**	62.20**	9.70**
Parents	12	62.19**	698.78**	1.26**	0.23**	36.09**	43.98**	8.56**
Females	7							
(Lines)		45.99**	708.63**	0.75**	0.17**	24.35**	37.96*	8.75**
Males	4							
(Testers)		99.23**	825.90**	2.36**	0.32**	65.06**	56.46**	7.80**
Female vs Male	1							
(Lines vs Testers)		27.46	121.40	0.42*	0.30*	2.44	36.24	10.28*
Hybrids	39	56.30**	539.25**	1.03**	0.20**	28.36**	61.46**	9.95**
Parent vs hybrid	1	3.53	3263.74**	6.38**	0.23*	87.13**	352.98**	17.72**
Check vs Hybrid	1	23.94	17.71	0.17	0.04	1.87	34.03	7.68
Error	106	12.94	156.82	0.10	0.04	2.75	14.62	2.16

*, ** were significant at 5% and 1% levels of probability, respectively.

Table Contd....

Table 1 *Contd...*

Source of variation	Df	Leaf width	Leaf: stem ratio	Green fodder yield per plant	Dry matter yield per plant	Dry matter content	Crude Protein content	Iron content
Replication	2	0.03	0.0004	394.89	11.41	6.70	0.21	21.31
Genotypes	53	0.20**	0.01**	2191.40**	157.41**	15.18**	2.84**	248.60**
Parents	12	0.17**	0.0088**	3199.27**	230.14**	13.20**	1.01**	129.02**
Females (Lines)	7	0.17**	0.0085**	2579.14**	193.76**	10.47**	0.60**	168.66**
Males (Testers)	4	0.19**	0.01**	4719.90**	330.84**	16.96**	1.85**	86.58*
Female vs Male (Lines vs Testers)	1	0.06	0.0005	1457.73*	81.97*	17.32*	0.58*	21.30
Hybrids	39	0.22**	0.01**	1945.88**	139.53**	16.46**	3.26**	288.99**
Parent vs hybrid	1	0.01	0.02**	1024.59*	64.84*	2.72	10.90**	317.76**
Check vs Hybrids	1	0.02	0.00	697.74	63.83*	1.16	0.13	0.23
Error	106	0.04	0.0026	219.36	14.82	2.71	0.13	33.48

*, ** were significant at 5% and 1% levels of probability, respectively

Table 2 : Summary of top five performing hybrids from forty crosses, in terms of desired standard heterosis; with their respective heterobeltiosis and mean data for 14 traits in forage pearl millet

Traits	Cross	Standard heterosis over GAFB-4 (%)	Heterobeltiosis (%)	Mean value of cross	Mean value of check (GAFB-4)	Reports reporting similar heterotic trends [#]
Days to flowering	AFB-67 × BAIF	-19.15**	-19.14**	50.67	62.67	Kapadiya. (2016); Ladumor <i>et al.</i> (2018)
	AFB-66 × AFB-45	-17.55**	-17.98**	51.67		
	AFB-25 × GIANT	-16.49**	-11.79*	52.33		
	BAJRA					
	AFB-24 × AFB-45	-14.89**	-4.19	53.33		
Plant height	AFB-23 × AFB-45	-14.89**	-17.94**	53.33		Vaghasiya <i>et al.</i> (2009); Chauhan <i>et al.</i> (2010); Maitry (2011); Kapadiya. (2016)
	AFB-23 × ICMO-1604	15.31**	19.12**	211.33 cm	183.27 cm	
	AFB-67 × GIANT	13.93**	23.25**	208.80 cm		
	BAJRA					
	AFB-23 × GIANT	13.71**	25.24**	208.40 cm		
Number of tillers per plant	AFB-67 × AFB-45	11.34**	12.70*	204.07 cm		Maitry (2011) and Jethva <i>et al.</i> (2012)
	AFB-25 × RBB-1	11.02**	0.98	203.47 cm		
	AFB-67 × ICMO-1604	56.86**	45.45**	5.33	3.40	
	AFB-23 × BAIF	45.10**	5.71	4.93		
	AFB-23 × AFB-45	37.25**	34.61**	4.67		
Stem diameter	AFB-66 × RBB-1	33.33**	65.85**	4.53		Kapadiya. (2016)
	AFB-24 × BAIF	29.41**	-5.71	4.40		
	AFB-25 × GIANT	-19.59**	-30.85**	2.11 cm	2.62 cm	
	BAJRA					
	AFB-23 × GIANT	31.60**	100.52**	25.27	19.20	
	BAJRA					Patel <i>et al.</i> (2008) and Maitry (2011)

Leaves per plant	AFB-24 × BAIF	25.00**	2.85	24.00			
	AFB-67× ICMO-1604	21.01**	17.73*	23.23			
	AFB-23 × BAIF	19.44**	-1.71	22.93			
	AFB-66 × RBB-1	17.01**	28.13**	22.47			
Leaf length	AFB-24 × AFB-45	17.57**	11.06*	70.93 cm	60.33 cm	Patel <i>et al.</i> (2008)	
	AFB-66 × BAIF	17.02**	13.99**	70.60 cm		Maitry (2011) and	
	AFB-44 × BAIF	16.46**	13.70**	70.27 cm		Kapadiya. (2016	
	AFB-67 × AFB-45	16.46**	10.02*	70.27 cm			
	AFB-23 × ICMO-1604	15.91**	13.52**	69.93 cm			
Internode length	No cross showed significant standard heterosis for this trait				19.17 cm	Vaghasiya <i>et al.</i> (2009) and Kapadiya. (2016)	
Leaf width	AFB-23 × ICMO-1604	28.50**	15.56*	3.37 cm	2.62 cm	Maitry (2011) and	
	AFB-67 × BAIF	24.43**	18.11**	3.26 cm		Kapadia (2016)	
	AFB-25 × BAIF	22.14**	6.19	3.20 cm			
	AFB-21 × ICMO-1604	20.36**	8.23	3.15 cm			
	AFB-44 × RBB-1	16.03*	16.32*	3.04 cm			
Leaf: Stem Ratio	AFB-25 × BAIF	20.89**	14.90*	0.81		Patel <i>et al.</i> (2008) and	
	AFB-23 × BAIF	19.40**	13.30*	0.80		Maitry (2011)	
	AFB-66 × BAIF	17.91**	12.16*	0.79	0.67		
	AFB-23 × GIANT BAJRA	17.91**	43.97**	0.79			
	AFB-24× ICMO-1604	14.92*	13.19*	0.77			
Green fodder yield per plant	AFB-66 × RBB-1	31.42**	105.85**	175.67 g	133.67 g	Ashwini <i>et al.</i> (2021) and Govindaraj <i>et al.</i> (2011) and Bhardwaj <i>et al.</i> (2023)	
	AFB-23 × GIANT BAJRA	24.94**	114.10**	167.00 g			
	AFB-23 × BAIF	20.20*	-3.40	160.67 g			
Dry matter yield per plant	AFB-66 × RBB-1	35.36**	129.67**	47.47 g	35.07 g	Govindaraj <i>et al.</i> (2011), Ashwini <i>et al.</i> (2021), and Bhardwaj <i>et al.</i> (2023)	
	AFB-23 × GIANT BAJRA	31.18**	130.00**	46.00 g			
Dry matter content	AFB-23 × AFB-45	21.76**	17.04**	32.03 %	26.31 %	Kapadia. (2016) and Ashwini <i>et al.</i> (2021)	
	AFB-66 × BAIF	10.90*	10.57*	29.18 %			
	AFB-67 × RBB-1	10.56*	11.78*	29.09 %			
	AFB-21 × ICMO-1604	42.84**	34.45**	10.67 %	7.47 %	Govindaraj <i>et al.</i> (2011), Ashwini <i>et al.</i> (2021) and Bhardwaj <i>et al.</i> (2023)	
Crude Protein content	AFB-24 × AFB-45	30.80**	10.56**	9.77 %			
	HC-20 × ICMO-1604	24.55**	5.68	9.30 %			
	HC 20 × GIANT BAJRA	23.66**	4.92**	9.23 %			
	AFB-44 × AFB-45	23.21**	8.23*	9.20 %			
Iron content	AFB-66 × ICMO-1604			11.46 mg/100g	10.06 mg/100g	Barathi and Reddy (2022)	
		13.92**	13.69**				
	AFB-67 × BAIF			11.42 mg/100g			
		13.52**	15.94**				
	AFB-23 × ICMO-1604			11.23 mg/100g			
		11.63**	10.75**				
	HC-20 × ICMO-1604			11.09 mg/100g			
		10.24**	11.01**				
	AFB-44 × BAIF			11.01 mg/100g			
		9.44**	25.97**				

*, ** were significant at 5% and 1% levels of probability, respectively

Note: In some traits, less than five crosses showed because standard heterosis was not found significant for other crosses

[#]: References listed in the rightmost column corroborate the presence, direction, and practical utility of significant heterobeltiosis and/or standard heterosis for the respective forage traits in pearl millet.

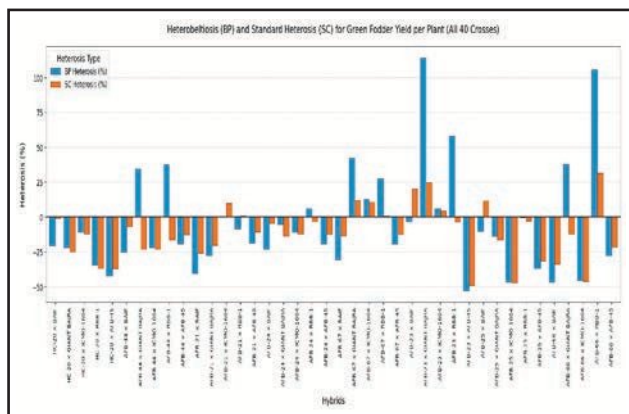


Figure 2: Population-wide distribution of heterobeltiosis (BP) and standard heterosis (SC) for green fodder yield per plant across all 40 F_1 hybrids, illustrating the range of heterotic responses beyond the top-performing crosses summarized in Table 2

In forage crop management, early flowering is a critical breeding objective because it dictates crop duration, facilitates timely green fodder availability, and allows seamless integration into intensive cropping sequences. Short-duration forage varieties enable livestock producers to bridge seasonal feed deficits during peak drought or transition periods. The analysis of variance revealed highly significant genetic variability for days to 50% flowering among the evaluated germplasm. In accordance with breeding objectives for maturity traits, substantial negative heterosis—indicative of dominance for early flowering alleles—was prominently observed across the hybrids. The cross AFB-67 $\times$ BAIF exhibited a highly significant standard heterosis of -19.15% over the commercial check GAFB-4, reaching 50% flowering in just 50.67 days compared to 62.67 days for the check. Similarly, AFB-66 $\times$ AFB-45 (-17.55%) and AFB-25 $\times$ GIANT BAJRA (-16.49%) demonstrated distinct phenological superiority by accelerating vegetative maturity by 10 to 11 days. This rapid transition is physiologically advantageous in rainfed *kharif* conditions, as it allows crops to escape terminal moisture stress while delivering early harvestable biomass—a trend that strongly corroborates the negative heterosis reported for flowering duration in pearl millet by Kapadiya (2016) and Ladumor *et al.* (2018).

Achieving high green fodder yield *per se* is insufficient for commercial forage evaluation, as the true nutritional and economic worth of fodder is governed by its dry matter content (DMC). Superior dry matter accumulation indicates a higher concentration of structural carbohydrates, soluble sugars, and essential minerals per unit of fresh weight rather than mere water retention. High dry matter content is vital for improving ruminant feed conversion ratios and ensuring optimum fermentation efficiency during silage and hay

preparation. The experimental material displayed substantial diversity for this trait, with the cross AFB-23 $\times$ AFB-45 emerging as an exceptional performer. This hybrid recorded 21.76% standard heterosis over GAFB-4 and 17.04% heterobeltiosis, achieving a dry matter density of 32.03% compared to the check's 26.31%. Other notable crosses, including AFB-66 $\times$ BAIF (10.90% standard heterosis) and AFB-67 $\times$ RBB-1 (10.56% standard heterosis), also demonstrated substantial improvements in dry matter accumulation. Such transgressive vigor in dry matter percentage reflects enhanced C_4 photosynthetic assimilation and rapid carbon partitioning—findings that align with the positive heterotic effects for dry matter yield previously reported by Kapadia (2016) and Ashwini *et al.* (2021).

In ruminant nutrition, morphological canopy traits—specifically the leaf-to-stem ratio (LSR), leaf length, and leaf width—serve as direct functional indicators of forage palatability, voluntary dry matter intake and organic matter digestibility. Because livestock prefer leafy fractions with lower lignin and neutral detergent fiber levels over fibrous stems, maximizing leafiness is a paramount objective in forage breeding. The present investigation revealed marked standard heterosis for canopy architectural traits. For leaf : stem ratio, AFB-25 $\times$ BAIF and AFB-23 $\times$ BAIF registered significant standard heterosis of 20.89% (0.81 vs. 0.67 check) and 19.40%, respectively, indicating a structural shift toward greater foliage proportion. Furthermore, significant horizontal and vertical leaf expansion was observed: AFB-23 $\times$ ICMO-1604 exhibited 28.50% standard heterosis for leaf width (3.37 cm), while AFB-24 $\times$ AFB-45 recorded 17.57% standard heterosis for leaf length (70.93 cm vs. 60.33 cm check). These architectural improvements drive higher photosynthetic surface area while simultaneously reducing overall mechanical resistance to grazing, corroborating the positive heterosis for leafiness and leaf : stem ratio documented by Patel *et al.* (2008), Maitry (2011), and Kapadia (2016).

Beyond vegetative bulk, biochemical quality dictates the commercial viability of forage pearl millet hybrids. A frequent challenge in forage breeding is the biomass dilution effect; wherein exceptionally high vegetative growth rates inversely reduce crude protein and mineral concentrations. However, this study demonstrates that selecting genetically diverse parental lines can effectively break this negative trade-off. For nutritional quality, the hybrid AFB-21 $\times$ ICMO-1604 exhibited a remarkable 42.84% standard heterosis for crude protein content, reaching 10.67% compared to 7.47% in GAFB-4. Similarly, for micronutrient density, AFB-66 $\times$ ICMO-1604 demonstrated 13.92% standard heterosis for iron content (11.46 mg/100g vs. 10.06 mg/100g check). Most notably,

the cross HC-20 × ICMO-1604 achieved dual nutritional superiority over the commercial check, recording both enhanced crude protein (9.30%; 24.55% standard heterosis) and elevated iron content (11.09 mg/100g; 10.24% standard heterosis). The ability to simultaneously enhance biomass density and protein-mineral accumulation through specific line-tester combinations mirrors the combining ability and heterotic patterns reported by Govindaraj *et al.* (2011), Ashwini *et al.* (2021), Barathi and Reddy (2022), and Bhardwaj *et al.* (2023).

A comparative synthesis of the top three performing crosses for green fodder yield per plant (Table 3) demonstrates that superior forage production is driven by distinct combinations of architectural and developmental traits. The hybrid 'AFB-66 × RBB-1' achieved the highest *per se* green fodder yield (175.70 g/plant), supported by a massive heterobeltiosis of 105.85% over its better parent and a highly significant standard heterosis of 31.42% over the commercial check GAFB-4. This yield superiority is primarily explained by substantial heterotic gains in vegetative bulk and biomass density, as the cross exhibited significant standard heterosis and heterobeltiosis for number of tillers per plant (33.33% and 65.85%, respectively), leaves per plant (17.01% and 28.13%, respectively), and dry matter yield per plant (35.36% and 129.67%, respectively). These results indicate that enhanced tiller density and leaf multiplication are the primary physiological drivers of fresh and dry matter

accumulation in this hybrid combination.

The second-highest yielding cross, 'AFB-23 × GIANT BAJRA' (167.00 g/plant), recorded 114.10% heterobeltiosis and 24.94% standard heterosis for green fodder yield. Its performance was characterized by pronounced vertical canopy growth and leafiness, showing significant desired standard heterosis for plant height (13.71%), leaves per plant (31.60%), leaf-to-stem ratio (17.91%), and dry matter yield per plant (31.18%). Notably, its high leaf-to-stem ratio (0.79) and significant heterobeltiosis across seven morphological and yield-contributing traits highlight its superior forage palatability and structural vigor.

Similarly, the cross 'AFB-23 × BAIF' (160.70 g/plant) demonstrated practical breeding utility by achieving a significant standard heterosis of 20.20% over GAFB-4, despite exhibiting a non-significant heterobeltiosis (-3.40%) for green fodder yield. This transgressive performance over the commercial check was driven by strong standard heterosis for number of tillers per plant (45.10%), leaves per plant (19.44%), leaf length (14.09%), and leaf-to-stem ratio (18.91%). Together, these three crosses illustrate that targeted parental selection can simultaneously improve vegetative yield, canopy architecture, and forage digestibility, making them prime candidates for top-cross evaluation, multi-location trials and transfer of male sterility system prior to commercial release.

Table 3: Comparison of top three promising crosses with high *per se* performance for green fodder yield per plant and their respective desired heterobeltiosis and standard heterosis for yield contributing characters

Hybrids	<i>Per se</i> performance (g)	Heterobeltiosis (%)	Standard heterosis (%)	Significant desired heterobeltiosis for yield contributing traits (%)	Significant desired standard heterosis for yield contributing traits (%)
AFB-66 × RBB-1	175.7	105.85**	31.42**	NTP (65.85**), LPP (28.13**), DMYP (129.67**), PH (25.24**), NTP (19.14*), LPP (100.52**), LW (15.36*), LSR (43.97**), DMYP (130.00**)	NTP (33.33**), LPP (17.01**), DMYP (35.36**)
AFB-23 × GIANT BAJRA	167.0	114.10**	24.94**	LL (11.38*), LSR (13.30*)	PH (13.71**), LPP (31.60**), LSR (17.91**), DMYP (31.18**), PH (8.51**), NTP (45.10**), LPP (19.44**), LL (14.09**), LSR (18.91**)
AFB-23 × BAIF	160.7	-3.40	20.20*		

*, ** were significant at 5% and 1% levels of probability, respectively.

PH : Plant height, NTP : Number of tillers per plant, LPP : Leaves per plant, LL : Leaf length, LW : Leaf width, LSR : Leaf: stem ratio, DMYP : Dry matter yield per plant, IC : Iron content

CONCLUSION

The present experiment focuses on the utility of heterosis for developing elite forage pearl millet hybrids with enhanced vegetative biomass and nutritional quality. Significant phenotypic variation across the evaluated genotypes confirms a broad genetic base suitable for targeted selection. Among the evaluated crosses, 'AFB-66 × RBB-1', 'AFB-23 × GIANT BAJRA', and 'AFB-23 × BAIF' emerged as top performers for overall green fodder production. Specific cross combinations also excelled in forage quality and adaptability namely, 'AFB-67 × BAIF' for early maturity, 'AFB-23 × AFB-45' for superior dry matter content and 'AFB-25 × BAIF' for enhanced leaf palatability traits. Nutritional superiority was highlighted by crude protein heterosis in 'AFB-21 × ICMO-1604', iron content heterosis in 'AFB-66 × ICMO-1604' and dual superiority over the commercial check for both crude protein and iron content in 'HC-20 × ICMO-1604'. Subject to validation in multi-location trials and transfer of sterility and restoration system, these elite, high-yielding, and nutritionally superior hybrids hold immense potential for commercial release, offering a viable strategy to bridge India's green fodder deficit and support the broader agricultural economy.

ACKNOWLEDGMENT

We are thankful to Sardarkrushinagar Dantiwada Agricultural University for financial support and smooth conduct of experiment.

REFERENCES

- Anonymous. (2024). *Basic animal husbandry statistics*. https://iss.icar.gov.in/Livestock_Documents/BAHS_2024.pdf
- AOAC. (2005). Official Methods of Analysis of AOAC INTERNATIONAL. https://www.researchgate.net/publication/292783651_AOAC_2005
- Ashwini, M. S.; Ganesan, K. N.; Ezhilarasi, T. and Sivakumar, S. D. (2021). Combining ability and heterosis studies in fodder pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Electronic Journal of Plant Breeding*, 12(3): 1011-1021.
- Barathi, M. B. and Reddy, P. S. (2022). Genetic analysis and heterosis for quality traits in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Electronic Journal of Plant Breeding*, 13(3): 838-844.
- Bhardwaj, R.; Singh, S.; Sohu, R. S. and Oberoi, H. K. (2023). Studies on combining ability and heterosis in three-way hybrids in forage pearl millet. *Range Management and Agroforestry*, 44(2): 255-262.
- Bhattacharya, A. and Mandal, A. B. (2003). Fodder yield and quality of some improved cultivars of pearl millet (*Pennisetum glaucum* L.). *Animal Nutrition and Feed Technology*, 3(1): 15-23.
- Chauhan, G. P.; Chovatiya, V. P.; Savaliya, J. J.; Mehta, D. R. and Pansurya, A. G. (2010). Heterosis studies in pearl millet [*Penisetum glaucum* (L.) R. Br.]. *Crop Research*, 39(1, 2 and 3): 98-102.
- Choudhary, S. (2023). Heterosis for grain yield and quality characters in pearl millet (*Pennisetum glaucum* (L.) Br.). *Annals of Plant and Soil Research*, 25: 157-162. <https://doi.org/10.47815/apr.2023.10250>
- Dhamodharan, P.; Bhuvaneshwari, J.; Sowmiya, S.; and Chinnadurai, R. (2024). Revitalizing fodder production: challenges and opportunities. *International Journal of Research in Agronomy*, 7:201-217.
- Fonseca, S. and Patterson, F. (1968). Hybrid vigour in a seven parent diallel crosses in common winter wheat (*Triticum aestivum* L.). *Crop Science*, 8(1): 85-88.
- Govindaraj, M.; Rai, K. N.; Kanatti, A.; Rao, A. S.; Shivade, H.; Dhanapal, A. P. and Blümmel, M. (2011). Pearl millet as a base for forage production: A review. *Tropical and Subtropical Agroecosystems*, 14(1): 231-242.
- Govintharaj, P.; Maheswaran, M.; Blümmel, M.; Sumathi, P.; Vemula, A. K.; Rathore, A.; Sivasubramani, S.; Kale, S. M.; Varshney, R. K. and Gupta, S. K. (2021). Understanding Heterosis, Genetic Effects, and Genome Wide Associations for Forage Quantity and Quality Traits in Multi-Cut Pearl Millet. *Frontiers in Plant Science*, 12: 1 - 13. <https://doi.org/10.3389/fpls.2021.687859>
- Jethva, A.S.; Raval, L.; Madariya, R.B.; Mehta, D.R. and Mandavia, C. (2012). Heterosis for grain yield and its related characters in pearl millet. *Electronic Journal of Plant Breeding*, 3(3):848-852.
- Kapadia, V. N. (2016). Estimation of Heterosis for Yield and Its Relevant Traits in Forage Pearl Millet [*Pennisetum glaucum* LR Br.]. *International Journal of Agriculture Sciences, ISSN*, 0975-3710
- Ladumor, V. L.; Mungra, K. D. and Parmar, S. K. (2018). Heterosis for grain yield components in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Trends in Biosciences*, 11(27): 3473-3477.

- Maitry G. (2011). Heterosis and combining ability in forage pearl millet [*Pennisetum glaucum* (L). R. Br.] Published M.Sc. thesis by Anand Agricultural University, Anand.
- Meredith, W. R. and Bridge, R. R. (1972). Heterosis and gene action in cotton [*G. Hirsutum* L.]. *Crop Science*. 12(3): 304-310
- Panse, V. G. and Sukhatme, P. V. (1985). Statistical Methods for Agricultural Workers (fourth edition), ICAR, New Delhi.
- Patel, M. A.; Yadavendra, J. P., Patel, D. H., Upadhyay, N. V. and Shaikh, J. A. (2008). Studies on heterosis for greenforage yield and its contributing traits in pearl millet [*Pennisetum glaucum* (L) R.Br.]. 34(3):149-155.
- Srivastava, R. K.; Bollam, S.; Pujarula, V.; Pusuluri, M.; Singh, R. B.; Potupureddi, G. and Gupta, R. (2020). Exploitation of Heterosis in Pearl Millet: A Review. *Plants*. 9: 807. <https://doi.org/10.3390/plants9070807>
- Vaghasiya, V. D.; Dangaria, C. J. and Dhedhi, K. K., (2009). Heterosis studies in B $\times$ R crosses for selection of superior females for A-line development in pearl millet. *Agricultural Science Digest*. 29(2): 84-88.
- Wang, C.; Guo, L.; Li, Y. and Wang, Z. (2012). Systematic comparison of C₃ and C₄ plants based on metabolic network analysis. *BMC systems biology*. 6(2): 1-14.
- Wheal, M. S.; Fowles, T. O. and Palmer, L. T. (2011). A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant tissue. *Analytical Methods*. 3(12): 2854–2863. <https://doi.org/10.1039/C1AY05430A>