

Assessment of variability for morphological, physiological and biochemical traits in pearl millet under rice fallow condition

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ABSTRACT

Rice fallows represent an underutilized agro-ecological niche with significant potential for crop diversification, especially through resilient, short-duration cereals like pearl millet (*Pennisetum glaucum* L. R. Br.). In this study, 20 pearl millet genotypes (17 hybrids and 3 checks) were evaluated under rice fallow conditions during the *Rabi* season of 2024–25 to examine variability in morphological, physiological and biochemical traits. The experiment revealed highly significant differences across all genotypes studied, indicating substantial genetic variability. High heritability coupled with high genetic advance was observed for grain yield, dry fodder yield, biomass, crop growth rate (CGR), zinc and iron content indicating additive gene action and strong potential for direct selection. Genotypes such as 291A × BL90 and 04999A × 1012 were performed over the checks in productivity and micronutrient content, suggesting their utility in biofortification and short-duration cropping. Traits like plant height, test weight and chlorophyll stability index showed moderate GCV and PCV with high heritability, indicating moderate improvement potential, while days to maturity and total phenol content showed moderate heritability but low genetic advance, reflecting non-additive gene action. The results highlight the scope for developing nutritionally enriched, high-yielding pearl millet hybrids tailored to rice fallow ecologies, contributing to food and nutritional security through sustainable intensification.

Keywords: Anti-nutritional factors, Heritability, Pearl Millet, Rice fallow and Variability

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is widely cultivated in tropical and subtropical regions. Its resilience to heat, water scarcity and poor soils makes it highly suitable for dryland farming (Varshney *et al.*, 2017). Globally, it ranks as the eighth most cultivated cereal and in India, it is the fifth major food grain. In 2023–24, India produced 107.16 lakh tonnes from 73.75 lakh hectares. Rajasthan is the leading state in area and production. Tamil Nadu recorded the highest productivity at 3,371 kg/ha (UPAG, 2023–24). Nutritionally, pearl millet is rich in starch, protein, dietary fibre and essential micronutrients like iron and zinc (Hassan *et al.*, 2021). However, antinutritional factors such as phytic acid and polyphenols limit its mineral bioavailability (Satyavathi *et al.*, 2017), necessitating breeding efforts to balance nutrition and agronomic performance. Post-*kharif* rice fallows remain underutilized in India, covering approximately 11.65 million hectares, including 1.1 million hectares in Andhra Pradesh (Srinivas *et al.*, 2022). Climatic challenges and pest pressures have reduced the

viability of traditional pulse crops in these areas, prompting interest in resilient, low-input alternatives. Pearl millet, with its short duration and stress tolerance, offers promising solution (Kouadio *et al.*, 2025).

To ensure its successful adoption in rice fallow ecosystems, development of genotypes tolerant to residual moisture and terminal drought is essential. This requires evaluating genetic variability for key agronomic and adaptive traits. The present study aims to assess the extent of variability through morphological, physiological and biochemical traits in pearl millet genotypes under rice fallow conditions to support region-specific breeding strategies.

MATERIALS AND METHODS

The present investigation was conducted at Agricultural College Farm, Bapatla, during *rabi* season, 2024–25 under rice fallow conditions. The experimental material consisted of 17 pearl millet genotypes and three checks—86M86, PRATAP, and ABH1200 obtained from ICAR–IIMR, Hyderabad

Table 1. The experiment was laid out in Randomized Block Design (RBD) with three replications. Each genotype was sown in four rows of three meters length, maintaining a spacing of 45 cm between rows and 10 cm between plants within the row. Standard agronomic practices were followed throughout the cropping period to ensure uniform crop growth and management.

Table 1. Pearl millet genotypes studied

S. No.	Genotype	S. No.	Genotype
1	04999A × 1413	11	291A × BL59
2	04999A × 1012	12	291A × BL90
3	84322A × 129R	13	291A × 1151R
4	221A × BL55	14	291A × BL57
5	221A × BL58	15	260A × BL53
6	269A × BL71	16	264A × BL58
7	269A × 1458	17	242A × 1352R
8	269A × 1526	18	86M86*
9	269A × 1449R	19	PRATAP*
10	291A × 2445R	20	ABH1200*

Observations were recorded on eight morphological traits, namely days to 50% flowering, days to maturity, plant height (cm), number of tillers per plant, ear head length (cm), grain yield (g/plant), dry fodder yield (g/plant) and 1000 grain weight (g). For physiological parameters, the Crop Growth Rate (CGR) was computed following the formula suggested by Watson (1952) and expressed in $\text{g/m}^2/\text{day}^{-1}$. Biomass accumulation was measured at harvest after drying the samples for one week and the mean values were recorded in kg/m^2 . Chlorophyll Stability Index (CSI) was estimated using the method proposed by Kaloyereas (1958) and expressed as a percentage.

Biochemical traits analyzed included seed iron and zinc content, total phenol content and phytic acid content. For mineral analysis, seeds were finely ground into flour and estimated for iron and zinc content as per the procedure of Tandon (1999), with results expressed in parts per million (ppm). Total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu reagent method as described by Singleton and Rossi (1965) and expressed as gallic acid equivalents (GAE). Phytic acid content was estimated following the method of Haug and Lantzsch (1983), with slight modifications and expressed in mg/g.

For statistical analysis, the analysis of variance (ANOVA) for each trait was carried out using the method proposed by Panse and Sukhatme (1957),

and significance was tested using F-values as per Fisher and Yates (1963). Estimates of genetic variability included phenotypic and genotypic coefficients of variation (PCV and GCV), calculated according to Burton (1952) and classified as per Sivasubramanian and Menon (1973). Broad-sense heritability (h^2_{bs}) was estimated following Lush (1940) and categorized using the classification proposed by Johnson *et al.* (1955). Genetic advance as a percentage of mean (GAM) was computed using the methodology of Lush (1940) and Johnson *et al.* (1955).

RESULTS AND DISCUSSION

Analysis of variance revealed that the mean sum of squares for all the traits exhibited highly significant differences, indicating substantial genetic variation among the genotypes under study Table 2. These significant differences across all the genotypes demonstrate the presence of considerable variability in the experimental material, suggesting the potential for effective selection and genetic improvement. Such variability is essential for identifying superior genotypes and enhancing crop performance through breeding programs. Similar results were reported by Sumathi *et al.* (2016), Govindaraj *et al.* (2020) and Rajpoot *et al.* (2023). The range and the mean performance of the genotypes for the studied traits are explained in Table 3.

Morphological traits

The mean performance of twenty pearl millet genotypes revealed considerable variability across key morphological traits, offering valuable insights for selection and improvement. Days to 50% flowering ranged from 44 to 54 days, with a mean of 50 days, reflecting noticeable differences in flowering behaviour and supporting classification into early and medium categories based on DUS guidelines. Days to maturity ranged between 73 and 83 days (mean: 79 days), indicating the availability of both early and late-maturing hybrids suitable for different cropping windows and agro-ecological conditions. Plant height showed a wide range from 118.2 cm to 172.9 cm, with taller hybrids like 291A × BL90 demonstrating potential for dual-purpose use, especially for biomass accumulation. The number of tillers per plant varied moderately (2.40 to 3.77), suggesting improved vegetative vigour and potential for higher yield. Ear

Table 2. Analysis of variance for morphological, physiological and biochemical characters

Source of variation	df	DF	DM	PH	NT	EHL	GY	DFY	TW
Mean sum of squares									
Replication	2	1.8	0.267	151.8	0.005	2.13	12.9	7.64	0.34
Genotypes	19	29.40**	33.87**	583.66**	0.560**	31.328**	79.38**	61.90**	5.09**
Error	38	0.853	1.02	72.19	0.029	1.81	8.28	4.53	1.18

Source of variation	df	CGR	CSI	BP	Zn	Fe	TPC	PA
Mean sum of squares								
Replication	2	1.144	0.898	0.001	11.28	0.8	0.212	0.068
Genotypes	19	161.08**	177.62**	0.206**	166.2**	293.85**	0.376**	4.617**
Error	38	6.905	6.24	0.002	12.52	29.51	0.1	0.112

****Level of Significance at 1%**

DF- days to 50% flowering, DM- Days to maturity, PH- plant height, NT- No. of tillers per plant, EHL- Ear head length, GY- Grain yield, DFY- Dry fodder yield, TW- Test weight, CGR- Crop growth rate, CSI- Chlorophyll stability index, BP- Biological production, Zn- Zinc content, Fe- Iron content, TPC- Total phenol content, PA- Phytic acid.

head length ranged from 16.5 cm to 26.8 cm, with longer panicles generally contributing to high grain yield. Grain yield itself varied substantially across genotypes, from 15.4 g to 31.5 g per plant, with the top-performing hybrids exhibiting 40–50% higher productivity than the best-performing checks, indicating strong heterosis and yield potential. Dry fodder yield ranged from 8.6 g to 24.8 g per plant, further reinforcing the value of these hybrids for integrated grain and fodder systems. Test weight, a key indicator of grain size and density, varied between 9.2 g and 15.3 g, with heavier grains associated with better seed quality and vigour. Overall, the observed variation across traits highlights the presence of promising hybrids with desirable agronomic characteristics, suitable for targeted cultivation and further genetic enhancement (Deewan *et al.*, 2023; Gowswami *et al.*, 2023; Yadav *et al.*, 2020).

Physiological traits

Significant variability was observed among the genotypes for crop growth rate (CGR), chlorophyll stability index (CSI) and biomass production, all of which are key physiological traits influencing crop productivity. CGR ranged from 22.74 to 47.26 g/m²/day, with an average of 35.57 g/m²/day (CV: 7.39%). The hybrid 291A × BL90 recorded the highest CGR, followed by 291A × BL57 and

291A × BL59, all of which outperformed the check varieties. CSI values ranged from 64.04% to 91.54%, with a mean of 76.15% (CV: 3.28%) indicating relatively stable expression across genotypes. Hybrids such as 291A × BL57 and 291A × BL90 maintained high CSI values, suggesting superior photosynthetic stability under stress conditions (Gunguniya *et al.*, 2023). Biomass production showed a mean of 0.97 kg/m² and ranged from 0.60 to 1.48 kg/m² (CV: 4.59%), with hybrids 291A × BL90 and 291A × BL59 recording the highest values. These hybrids demonstrate potential for dual-purpose cultivation, contributing not only to grain yield but also to fodder availability thereby enhancing their suitability for integrated crop-livestock systems. Overall, the results highlight the physiological robustness of select hybrids and their suitability for sustainable intensification under resource-constrained environments.

Biochemical traits

Significant variability was observed among the pearl millet genotypes for key biochemical traits, indicating their potential utility in biofortification and nutritional improvement programs. Zinc content ranged from 32.33 to 57.57 ppm, with a mean value of 46.72 ppm and a coefficient of variation (CV) of 7.58%. Notably, hybrids such as 04999A × 1012, 264A × BL58 and 291 × 1151R exhibited significantly higher

zinc concentrations than the standard checks, making them promising candidates for zinc enrichment. These results are consistent with earlier findings reported by Singh *et al.* (2024) and Gangashetty *et al.* (2023), who also identified genotypes with enhanced zinc accumulation.

Iron content showed considerable variation, ranging from 43.93 to 84.37 ppm (mean: 67.09 ppm; CV: 8.10%). Hybrids 291A × BL57, 04999A × 1012 and 291A × 1151R demonstrated superior iron concentrations compared to checks, underscoring their potential for iron biofortification. These findings are in agreement with Gunguniya *et al.* (2023) and Gangashetty *et al.* (2023), who emphasized the importance of genetic variation in improving iron density in pearl millet.

Total phenol content (TPC) among the genotypes varied from 2.54 to 3.91 mg GAE/g, with an average of 3.08 mg and a CV of 10.27%. Hybrids such as 291A × BL90 and 221A × BL58 recorded lower phenol content than the checks, indicating a reduced antinutritional load, which is desirable for enhancing nutrient absorption. These observations align with those of Salar and Purewal (2016) and Meena *et al.* (2018), who highlighted the breeding value of genotypes with low phenolic content.

Phytic acid content ranged from 5.53 to 9.66 mg/g (mean: 7.76 mg; CV: 4.32%). Hybrids 04999A × 1012 and 291 × 1151R recorded lower phytic acid levels than the checks, suggesting improved mineral bioavailability. These results corroborate previous reports by Singh *et al.* (2024) and Gunguniya *et al.* (2023), reinforcing the role of such genotypes in developing nutritionally enhanced pearl millet varieties.

Genetic Variability Studies

Phenotypic and Genotypic Coefficients of Variation

The assessment of PCV and GCV for 15 traits revealed varying degrees of genetic variability among the evaluated pearl millet genotypes Table 4. Traits such as days to 50% flowering, days to maturity, and plant height exhibited low PCV and GCV, indicating limited genetic variability (Anuradha *et al.*, 2020; Deewan *et al.*, 2023; Rani *et al.*, 2019). Similarly, Chlorophyll Stability Index (CSI) also showed low GCV (9.93%) and moderate PCV (10.45%) (Pavithra *et al.*, 2024). Moderate PCV

and GCV were recorded for traits like number of tillers per plant, ear head length, test weight, zinc and iron content, phytic acid and crop growth rate implying moderate genetic variability and good prospects for selection (Dapke *et al.*, 2014; Chauhan *et al.*, 2020; Asungre *et al.*, 2021). Total phenol content also fell into this category, with moderate PCV (14.24%) but low GCV (9.87%), suggesting environmental influence slightly exceeds genetic control (Meena *et al.*, 2018). Traits such as grain yield, dry fodder yield and biomass production exhibited high PCV and GCV, highlighting substantial genetic variability and a strong genetic basis for these economically important traits (Kumar *et al.*, 2020; Yadav *et al.*, 2020). The narrow differences between PCV and GCV in most traits indicated that environmental effects were relatively low and that observed variability was primarily due to genetic factors.

Heritability (h^2_{bs}) and Genetic advance as percent of mean (GAM)

The analysis of heritability and genetic advance revealed distinct patterns of gene action across traits in pearl millet. Traits such as number of tillers per plant (heritability: 85.75%, genetic advance: 26.86%), ear head length (84.43%, 27.48%), grain yield (74.10%, 38.34%), dry fodder yield (80.88%, 51.99%), crop growth rate (88.15%, 38.98%), biological production (97.19%, 54.83%), zinc content (80.36%, 28.29%), iron content (74.91%, 24.94%) and phytic acid content (93.05%, 31.38%) showed high heritability coupled with high genetic advance. This combination suggests the predominance of additive gene action and minimal environmental influence, making these traits highly responsive to direct selection (Kumar *et al.*, 2020; Yadav *et al.*, 2020; Singh *et al.*, 2024). Traits like days to 50% flowering (91.78%, 12.19%), plant height (70.25%, 15.80%) and chlorophyll stability index (90.15%, 19.41%) exhibited high heritability but only moderate genetic advance, indicating both additive and non-additive gene effects and suggesting that moderate progress can be achieved through selection, possibly enhanced *via.*, recombination-based approaches (Chauhan *et al.*, 2020).

In contrast, days to maturity (91.47%, 8.26%) had high heritability but low genetic advance, implying the dominance of non-additive gene action and limiting the effectiveness of direct selection—hence, heterosis breeding may be more appropriate

Table 3. Mean performance of the pearl millet genotypes for the traits

Genotypes	DF	DM	PH		NT	EHL (cm)	GY		DFY	TW (g)	CGR (g m ⁻² day ⁻¹)	CSI (%)	BP		Zn (ppm)	Fe (ppm)	TPC (mgGAE/g)	PA (mg/g)
			(cm)	(cm)			(g/plant)	(g/plant)					(Kg/m ²)	(Kg/m ²)				
04999A×1413	49	77	137.1	2.6	2.6	18.23	15.43	12.1	9.2	30.82	68.76	0.61	52.17	77.1	3.03	7.01		
04999A×1012	50	77	135	2.4	2.4	18.15	16.93	10.56	12.37	25.7	64.04	0.79	57.57	77.9	3.11	5.53		
843-22A×129R	44	73	136.1	2.6	2.6	18.23	20.11	12.11	11.57	29.68	81.59	0.76	42.6	55.43	2.92	5.53		
221A×BL55	49	78	140	2.5	2.5	21.53	21.24	14.15	12.37	33.4	74.2	1.13	44.9	60.5	2.62	9.27		
221A×BL58	51	82	148.2	2.5	2.5	25.95	30.65	19.95	15.3	42.46	76.36	0.99	32.33	43.93	2.65	6.59		
269A×BL71	49	79	119.7	3.3	3.3	23	18.95	11.59	12.07	31.33	70.71	1.18	48.03	67.9	3.44	8.93		
269A×1458	50	79	143.7	3.5	3.5	22.13	22.73	15.4	12.27	38.49	76.32	0.97	32.9	55.5	3.13	6.56		
269A×1526	53	82	128.7	3.2	3.2	24.47	20.59	13.27	10.97	38.77	73.27	0.85	40.33	72.9	3.32	8.11		
269A×1449R	52	82	151.3	3.6	3.6	25.73	25.44	18.48	12.37	42.8	71.96	1.16	38.93	60.23	3.15	8.79		
291A×2445R	54	83	167.3	2.7	2.7	22.07	29.64	20.68	12.87	38.99	78.97	1.23	46.93	61.33	2.79	8.65		
291A×BL59	52	81	156.1	3.8	3.8	26.81	28.17	23.25	12.3	42.92	83.93	1.43	48	72.7	3.13	9.65		
291A×BL90	53	83	172.9	3.7	3.7	26.55	31.53	24.77	13.97	47.26	84.49	1.48	52.5	61.63	2.54	8.79		
291A×1151R	52	81	149.1	2.7	2.7	22.15	20.12	15.62	12.53	38.32	71.12	0.67	54.97	75.53	3.39	6.1		
291A×BL57	54	83	158.3	3.4	3.4	21.23	28.61	19.65	10.37	45.17	91.54	0.62	42.4	84.37	3.39	8.18		
260A×BL53	45	74	137.3	3.2	3.2	22.99	19.82	13.74	12.27	26.66	64.86	0.84	46.83	65.73	3.44	7.24		
264A×BL58	51	80	135.2	2.8	2.8	21.1	25.97	17.82	13.8	38.49	69.78	0.88	55.67	68.35	3.26	8.94		
242A×1352R	46	76	135.9	3.1	3.1	16.52	15.46	9.61	11.33	22.74	68.87	1.18	38.97	64.93	3.91	7.77		
86M86	51	81	143.7	2.7	2.7	19.89	22.15	17.82	13.33	37.09	84.01	1.09	50.03	78.17	2.75	7.36		
PRATAP	49	74	139.7	2.6	2.6	17.55	21	12.54	12.03	37.55	80.61	0.86	53.17	61.4	2.61	8.66		
ABH1200	44	74	118.2	2.9	2.9	17.44	15.85	8.65	11.97	22.79	87.63	0.6	55.07	76.33	2.96	7.5		
Mean	50	79	142.7	3	3	21.59	22.52	15.59	12.26	35.57	76.15	0.97	46.72	67.09	3.08	7.76		
Min	44	73	118.2	2.4	2.4	16.52	15.43	8.65	9.2	22.74	64.04	0.6	32.33	43.93	2.54	5.53		
Max	54	83	172.9	3.8	3.8	26.81	31.53	24.77	15.3	47.26	91.54	1.48	57.57	84.37	3.91	9.66		
CV (%)	1.8	1.3	5.96	5.7	5.7	6.24	12.78	13.65	8.86	7.39	3.28	4.59	7.58	8.1	10.27	4.32		
SEm	0.5	0.6	4.91	0.1	0.1	0.78	1.66	1.23	0.63	1.52	1.44	0.03	2.04	3.14	0.18	0.19		
CD @ 5%	1.5	1.7	14.04	0.3	0.3	2.23	4.76	3.52	1.8	4.34	4.13	0.07	5.85	8.98	0.52	0.55		

DF- days to 50% flowering, DM- Days to maturity, PH- plant height, NT- No. of tillers per plant, EHL- Ear head length, GY- Grain yield, DFY- Dry fodder yield, TW- Test weight, CGR- Crop growth rate, CSI-Chlorophyll stability index, BP- Biological production, Zn- Zinc content, Fe- Iron content, TPC- Total phenol content, PA- Phytic acid

Table 4: Estimates of genetic parameters of pearl millet genotypes

S.No.	Traits	GCV (%)	PCV (%)	h^2b (%)	GAM (%)
1	Days to 50% flowering	6.18	6.45	91.78	12.19
2	Days to maturity	4.19	4.38	91.47	8.26
3	Plant Height	9.15	10.92	70.25	15.8
4	No of tillers per plant	14.08	15.21	85.75	26.86
5	Ear head length	14.52	15.8	84.43	27.48
6	Grain yield	21.62	25.12	74.1	38.34
7	Dry fodder yield	28.07	31.21	80.88	52
8	Test weight	9.32	12.86	52.53	13.92
9	Crop growth rate	20.15	21.47	88.16	38.98
10	Chlorophyll stability index	9.93	10.45	90.15	19.41
11	Biomass production	27	27.39	97.19	54.83
12	Iron	15.32	17.09	80.36	28.3
13	Zinc	13.99	16.17	74.91	24.95
14	Total phenols	9.87	14.24	48	14.08
15	Phytic acid	15.79	16.37	93.05	31.38

(Govindaraj *et al.*, 2010; Kumar *et al.*, 2020). Traits such as test weight (52.53%, 13.91%) and total phenol content (48.01%, 14.08%) showed moderate heritability and genetic advance, indicating mixed genetic control and modest potential for early-generation selection. Overall, the findings underscore that most productivity and nutritional traits in pearl millet are primarily governed by additive gene effects, making them strong candidates for genetic improvement through direct selection, while a few traits influenced by complex gene interactions may require alternative breeding strategies for effective enhancement.

CONCLUSION

The study under rice fallow conditions revealed substantial genetic variability among pearl millet genotypes, offering valuable avenues for crop improvement. Traits such as grain yield, dry fodder yield, biomass, CGR, zinc and iron content exhibited high heritability and genetic advance, suggesting additive gene action and strong prospects for direct selection. Genotypes like 291A × BL90 and 04999A × 1012 emerged as superior for grain yield and micronutrient enrichment. Moderate gains in plant height, test weight, and CSI indicate potential for multi-trait selection. Limited progress in days to maturity and TPC suggests a need for heterosis breeding. These findings support targeted breeding for rice fallow adaptation.

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