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Genetic variability and trait associations for improved yield and rancidity in pearl millet

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Abstract
The 100-pearl millet inbred lines (75 R-lines and 25 B-lines) were evaluated in alpha lattice design during *Kharif* 2023 at two locations (ARS, Perumallapalle, Tirupati, Andhra Pradesh and ICAR-IIMR, Hyderabad, Telangana). The genetic parameters were estimated among the lines and interrelationships were assessed for nine agronomic and yield traits and rancidity. The analysis of variance revealed significant differences among inbred lines for all the traits across the locations indicating the presence of considerable amount of genetic variability in the material. High GCV and PCV were observed for alcoholic acidity and high heritability coupled with high genetic advance as percent of mean observed for number of productive tillers plant^{-1} and alcoholic acidity indicated the presence of additive genetic effects. Significant positive association of grain yield with panicle weight, panicle length and panicle girth and significant negative association with alcoholic acidity indicates the possibility of deployment of low rancidity in high yielding background.

Keywords: Pearl millet, genetic variability, heritability, correlation analysis, grain yield and alcoholic acidity

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br], a diploid species (2n = 14), originated in West Africa is a vital staple crop particularly for people residing in arid and semi-arid regions due to its exceptional drought tolerance, high nutritional value and adaptability to harsh environmental conditions. Pearl millet caters the nutritional needs of households, earning its moniker "Nutri cereals" due to its outstanding nutritional profile. It is the sixth most important cereal crop in world next to maize, rice, wheat, barley and sorghum in terms of global area (30 m ha) and production (28 Mt) with majority of area in Asia (18 m ha) and Africa (10 m ha). Pearl millet grains are of good nutritive value with 73% carbohydrates, 11% protein, 8.4% fat and rich source of minerals such as iron (6-7 mg/100 g) and zinc (3.4 mg/100 g) (Bhargavi *et al.,* 2024) [3]. Despite its excellent nutritional value, it has a low shelf life due to the rapid development of rancidity and hence there is a need to develop cultivars with high nutrient contents, low anti-nutritional factors along with more grain yield.

As an important food and fodder crop, improving its grain yield and associated agronomic traits remains a key objective in breeding programs. Assessing genetic parameters is important for the better exploitation of heterosis available in the base material for grain yield and yield attributes. Knowledge on genetic variability parameters such as genotypic coefficient of variation, heritability and genetic advance helps breeders identify promising traits with a high potential for genetic gain. High heritability estimates together with high genetic advance are more valid for selection than heritability estimates alone (Johnson *et al.,* 1955) [9]. Estimation of genetic variability along with heritability and genetic advance gives an idea of the possible improvement of the trait through selection.

In a breeding program, the selection of superior genotypes primarily focuses on enhancing yield, which is crucial for improving productivity and overall crop performance. Yield and its contributing traits are complex and often interdependent, necessitating an understanding of their relationships and their direct and indirect effects on yield. Correlation analysis aids in identifying key traits and trait combinations that can be targeted through selection to enhance grain yield.

Considering this, the present study was conducted to assess genetic parameters, interrelationships among yield-related traits and their impact on grain yield, including alcoholic acidity in pearl millet.

Materials and Methods

A set of 100 pearl millet inbred lines comprising 75R-lines (restorer lines) and 25 B-lines (maintainer lines) of diverse pedigree procured from pearl millet breeding unit, ICAR-Indian Institute of Millets research, Hyderabad, India was used in the present study. The experiment was carried out in alpha lattice design with two replications during *Kharif* 2023 (June to October) at two locations *viz.,* Agricultural Research Station, Perumallapalle, Tirupati (altitude of 182.9 m MSL, 13°N latitude and 79° E longitude) and ICAR-Indian Institute of Millets Research (ICAR-IIMR), Rajendranagar, Hyderabad (altitude of 476.5 m MSL, 17°04' N latitude and 75°54' E longitude). Each replication of experiment contains 10 blocks with 10 plots pear each block. Each entry was sown in 2 rows of 2m length with a spacing of 15 cm between plant to plant at uniform depth and 60 cm between the rows at both the locations. All recommended package of practices were followed to raise the healthy crop. Biometric traits such as days to 50% flowering and days to maturity were assessed at the plot level, while plant height (cm), number of productive tillers plant^{-1}, panicle length (cm), panicle girth (cm), panicle weight (g), 1000 seed weight (g), grain yield plant^{-1} and alcoholic acidity were recorded based on observations from five randomly selected plants.

Statistical Analysis

Analysis of variance was worked out following the method suggested by Patterson and Williams (1976) [17] and the genotypic and phenotypic coefficient of variations were estimated by the method suggested by Burton (1952) [5] and heritability (broad sense) as suggested by Lush (1940). The procedure suggested by Johnson *et al.* (1955) [9] was followed for calculating the genetic advance and genetic advance as percent of mean. Correlation coefficients were calculated using the formula proposed by Singh and Choudhary (1977) [24]. All statistical analysis and the correlation plot were generated using R software version 4.4.1.

Results and Discussion

Analysis of variance

Analysis of variance for individual locations revealed significant differences among 100 inbred lines (75 B-lines and 25 R-lines) for 10 traits under study indicating the presence of considerable amount of genetic variability in the material in both locations *i.e.,* Tirupati and Hyderabad. Pooled ANOVA across locations revealed significant variation due to genotypes and due to environment for all the traits and due genotype × environment interaction for all the traits except days to 50% flowering, days to maturity, number of productive tillers plant^{-1}, and 1000 seed weight indicating that the trait expression is influenced not only by the genetic makeup of the inbreds but also by the environment conditions across the locations (Table 1).

Variability studies and Genetic Parameters

Success of any hybrid development program largely depends on the selection of elite parental inbreds. Selection of superior inbreds will be possible only when adequate variability exists in the gene pool which is of greatest interest to the plant breeder as it plays a vital role in framing successful breeding programme (Wedwesen and Zeleke, 2020) [28]. Higher the amount of variation present for a character in the breeding material, greater is the scope for its improvement through selection.

The mean and range of 100 pearl millet inbred lines for 10 traits across the locations is presented in Table 2 and Figure 1, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2_b) and genetic advance as percent of mean across the locations are presented in Table 2. The phenotypic coefficient of variation was of higher magnitude than genotypic coefficient of variation for all the traits except alcoholic acidity indicating the influence of environment on the expression of these traits.

Of all the traits, only alcoholic acidity showed high GCV (28.64%) and high PCV (28.64%) indicating the ample amount of genetic variation among the inbred lines and simple selection would be effective for further improvement of this trait. The traits *viz.,* number of productive tillers plant^{-1} (GCV: 16.16%; PCV: 20.89%), grain yield plant^{-1} (GCV: 14.27%; PCV: 25.54%), 1000 seed weight (GCV: 11.76%; PCV: 16.67%) panicle weight (GCV: 11.70%; PCV: 24.37%), plant height (GCV: 11.18%; PCV: 17.58%) and panicle girth (GCV: 10.11%; PCV: 13.89%) showed moderate estimates of GCV and PCV. While the traits *viz.,* plant height (GCV: 11.18%; PCV: 17.58%), days to 50% flowering (GCV: 5.26%; PCV: 7.23%) and days to maturity (GCV: 3.82%; PCV: 4.92%) showed lower estimates of GCV and PCV suggesting the little scope for further improvement of these characters through selection.

Similar results of GCV and PCV in pearl millet inbreds were earlier reported by Pallavi *et al.* (2023) [14] and Bhargavi *et al.* (2024) [3] for alcoholic acidity, Sumathi *et al.* (2016) [26], Basavaraj *et al.* (2017) [2], Solanki (2017) [25], Ramya *et al.* (2018) [19] and Kumawat *et al.* (2019) [11] for grain yield plant^{-1} and 1000-grain weight, Sharma *et al.* (2018) [21, 22] for number of productive tillers plant^{-1}, Gugarwal (2019) [8] and Patel *et al.* (2019) [15] for panicle length and panicle girth, Vagadiya *et al.* (2013) [27], Sharma (2018) [21, 22] and Kumawat *et al.* (2019) [11] for days to 50% flowering, Govindaraj *et al.* (2011) [6], Singh *et al.* (2014) [23] and Saikumar *et al.* (2020) [20] for days to maturity.

The knowledge on heritability (h^2_b) enables the plant breeder to decide the course of selection procedure to be followed under a given situation. Selection made on the basis of heritability alone is likely to be misleading and it becomes necessary to determine the parameters under targeted production environment. Thus, selection of traits based on heritability and genetic advance as percent of mean is more reliable in development of effective selection criteria for trait improvement (Johnson *et al.,* 1955) [9].

High heritability coupled with high genetic advance as percent of mean (GAM) was observed for alcoholic acidity (h^2_b = 95.24%, GAM = 59.00%) and number of productive tillers plant^{-1} (h^2_b = 60.90%, GAM = 25.77%) indicating the preponderance of additive gene action and therefore direct selection for these traits would be effective for developing low rancid lines. Moderate heritability with moderate genetic advance as percent of mean was observed for panicle girth (h^2_b = 53.00%, GAM = 15.16%), 1000 seed weight (h^2_b = 49.70%, GAM = 17.08%), panicle length (h^2_b

= 33.30%, GAM = 10.93%), grain yield plant^{-1} (h^2_b = 31.20%, GAM = 16.43%) and plant height (h^2_b = 21.05%, GAM = 14.64%). These findings of high heritability with high genetic advance as percent of mean were earlier reported by Lakshmana *et al.* (2009) [12], Govindaraj *et al.* (2011) [6] and Saikumar *et al.* (2020) [20] for number of productive tillers plant^{-1} and Pallavi *et al.* (2023) [14] Bhargavi *et al.* (2024) [3] for alcoholic acidity. While moderate estimates of both heritability and genetic advance as percent of mean was earlier reported by Kumari and Nagarajan (2008) [10] and Vagadiya *et al.* (2013) [27] for panicle girth, panicle length and 1000 seed weight and Yahaya (2015) [29] for grain yield plant^{-1}.

Correlation Coefficient Analysis

Grain yield is a complex character which is highly influenced by the environment and is the result of interrelationships of various yield components (Grafius, 1960) [7]. Understanding the phenotypic and genotypic correlation coefficients among various plant traits helps to ascertain the degree to which these are associated with economic productivity. Phenotypic correlation measures the extent to which two traits are linked due to both genetic and environmental factors, whereas genotypic correlation specifically quantifies the genetic association between traits. Evaluating these correlations within and across variables in a crop breeding program facilitates indirect selection (Pavan *et al.,* 2011) [18]. Hence, a sound knowledge of traits association is necessary in order to select the traits for developing high yielding hybrids.

Phenotypic and genotypic correlations between grain yield plant^{-1} and yield traits along with alcoholic acidity across a group of 100 pearl millet inbred lines pooled across the locations are presented in Table 3 and Figure 2. These results revealed that genotypic correlation coefficients were higher than the phenotypic correlation coefficients indicating the strong inherent association between traits studied and its phenotypic expression. In the present study, the traits *viz.,* panicle weight (r_g = 0.979**, r_p = 0.833**) followed by panicle length (r_g = 0.596**, r_p = 0.482**), panicle girth (r_g = 0.426**, r_p = 0.375**), plant height (r_g = 0.276**, r_p = 0.109**), days to 50% flowering (r_g = 0.276**, r_p = 0.064*) and days to maturity (r_g = 0.247**, r_p = 0.065*) recorded significant positive association with grain yield plant^{-1} at both phenotypic and genotypic levels indicating scope for their simultaneous improvement with grain yield plant^{-1}. Similar outcomes were earlier reported by Bhasker *et al.* (2017) [4], Kumawat *et al.* (2019) [11], Annamalai *et al.* (2020) [1] and Patil *et al.* (2021) [16]. In contrast, alcoholic acidity exhibited significant negative association with grain yield plant^{-1} (r_g =-0.225**, r_p =-0.079*) in desirable direction indicating selection for higher yield may concurrently reduce alcoholic acidity, leading to improved grain quality and storage stability.

For an effective breeding strategy, in addition to identification of yield attributing traits showing positive and significant association with grain yield, the information about the association of yield attributes with each other is also essential. Understanding these associations helps in selecting key traits that contribute to yield improvement. Interrelationship between yield characters revealed that plant height had significant positive association with panicle length (r_g = 0.493**, r_p = 0.349**), panicle length with panicle weight (r_g = 0.601**, r_p = 0.475**), panicle girth with panicle weight (r_g = 0.399**, r_p = 0.412**) highlighting the role of panicle robustness in determining grain yield plant^{-1}. Days to 50 % flowering had highly significant positive association with days to maturity (r_g = 0.994**, r_p = 0.934**) and negative association with number of productive tillers plant^{-1} (r_g =-0.443**, r_p =-0.270**) and thousand seed weight (r_g =-0.308**, r_p =-0.183**). Further alcoholic acidity exhibited significant negative association plant height (r_g =-0.188**, r_p =-0.081) and panicle length (r_g =-0.151**, r_p =-0.058) suggesting that the negative association of these traits would be more advantageous when selecting low rancid inbred lines with high yield contributing traits.

Table 1: Analysis of Variance for yield and its component traits along with alcoholic acidity among 100 pearl millet inbred lines

Location	Source of Variation	d.f	DFF	DM	PH	NPT	PL	PG	PW	TSW	GYP	AA
Tirupati-*Kharif* 2023	Replication	1	1.28	12.01	1336.45	0.015	5.70	0.39	17.67	1.67	4.15	0.31
	Genotype	99	35.85**	41.54**	1286.52**	0.50**	21.54**	0.23**	161.33**	6.25**	73.82**	0.003**
	Replication: Block	9	17.71	17.05	417.61	0.03	8.45	0.15	112.43	1.53*	13.95	0.001
	Error	90	21.49	22.09	340.86	0.02	11.72	0.13	78.94	0.75	37.97	0.0002
Hyderabad-*Kharif* 2023	Replication	1	0.08	13.00	272.22	2.34*	10.39	0.57**	71.34	0.76	54.01	0.31
	Genotype	99	35.38**	55.97**	939.14*	0.30*	15.35*	0.30**	53.23**	6.70**	26.79**	0.005**
	Replication: Block	9	6.31	8.77	568.55	0.35	9.63	0.03	52.89	3.91	20.02	0.0002
	Error	90	9.03	14.92	677.68	0.20	9.99	0.06	36.68	3.62	18.53	0.0002
Pooled-*Kharif* 2023	Genotype	99	26.38**	13.6**	19507.8**	1.90**	15.83**	0.03**	105.6**	47.14**	240.5*	0.001**
	Environment	1	2658.16**	6443.5**	5510.5**	6.51**	79.26*	0.99*	31231.1**	372.88**	11999.5**	0.00004
	Genotype: Environment	99	9.71	1.9	14.2**	0.02	3.02**	0.29**	43.1**	0.71	5.9**	0.001
	Block: Replication	9	22.70	66.3	380.9	0.26	13.86	0.55	101.5	2,29	96.1	0.17
	Environment: Replication	2	2.21	0.91	2551.9	0.15	13.06	0.16	57.6	0,73	2.2	0.19
	Environment: Block: Replication	18	9.26	52.7	205.8	0.21	2.50	0.16	31.00	0,02	35.1	0.03
	Error	392	25.00	33.1	762	0.26	14.58	0.18	83.6	4.28	38.4	0.003

* : Significant at 5% level; ** : Significant at 1 % level

DFF : Days to 50% flowering	NPT : Number of productive tillers plant^{-1}	PW : Panicle weight	AA : Alcoholic acidity
DM : Days to maturity	PL : Panicle length	TSW : Thousand seed weight	
PH : Plant height	PG : Panicle girth	GYP : Grain yield plant^{-1}	

Table 2: Estimates of variability and genetic parameters for yield traits and alcoholic acidity among 100 pearl millet inbred lines across the locations

Trait	Mean	Range		Variance			Coefficient of Variation			Heritability (Broad sense) (%)	Genetic advance (GA)	Genetic advance as per cent of mean (%)
		Min.	Max.	Genotypic	Phenotypic	Environmental	Genotypic	Phenotypic	Environmental			
DFF	55.57	44.50	63.00	8.54	16.15	7.61	5.26	7.23	4.96	52.90	4.38	7.88
DM	92.89	80.00	101.00	12.60	20.84	8.25	3.82	4.92	3.09	60.40	5.68	6.12
PH	143.85	80.32	199.73	258.56	639.80	381.24	11.18	17.58	13.57	40.40	21.05	14.64
NPT	1.91	1.17	2.75	0.10	0.16	0.06	16.16	20.89	13.23	60.90	0.49	25.77
PL	21.23	13.68	27.37	3.82	11.47	7.66	9.20	15.95	13.03	33.30	2.32	10.93
PG	2.64	1.81	3.69	0.07	0.13	0.06	10.11	13.89	9.52	53.00	0.39	15.16
PW	29.97	18.74	43.57	12.30	53.32	41.02	11.70	24.37	21.37	23.10	3.47	11.58
TSW	8.99	5.92	13.44	1.12	2.25	1.13	11.76	16.67	11.82	49.70	1.53	17.08
GYP	19.49	9.08	31.00	7.74	24.79	17.05	14.27	25.54	21.18	31.20	3.20	16.43
AA	0.16	0.08	0.30	0.002	0.002	0.0001	28.64	28.64	7.71	95.24	0.08	59.00

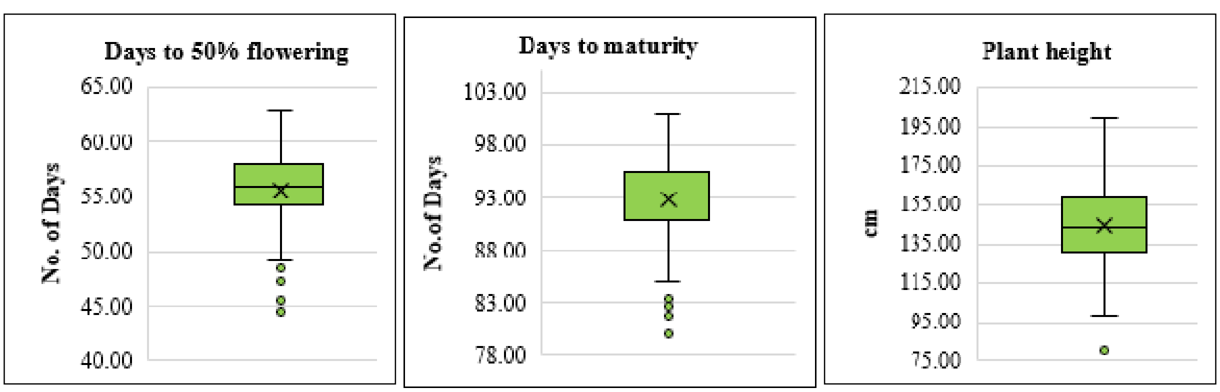
DFF : Days to 50% flowering	NPT : Number of productive tillers plant^{-1}	PW : Panicle weight	AA : Alcoholic acidity
DM : Days to maturity	PL : Panicle length	TSW : Thousand seed weight	
PH : Plant height	PG : Panicle girth	GYP : Grain yield plant^{-1}	

Table 3: Phenotypic (r_p) and Genotypic (r_g) correlation coefficients among yield, yield attributes and alcoholic acidity in 100 pearl millet inbred lines across the locations

		DFF	DM	PH	NPT	PL	PG	PW	TSW	AA	GYP
DFF	r_p	1.000	0.934**	0.001	-0.270**	0.120*	0.188*	0.169*	-0.183*	0.178*	0.064*
	r_g	1.000	0.994**	-0.016	-0.443**	0.136*	0.218**	0.305**	-0.308**	0.251**	0.276**
DM	r_p		1.000	0.019	-0.252**	0.131*	0.182*	0.172*	-0.219**	0.181*	0.065*
	r_g		1.000	-0.016	-0.391**	0.118*	0.231**	0.375**	-0.384**	0.233**	0.247**
PH	r_p			1.000	-0.131*	0.349**	-0.146*	0.167*	-0.073	-0.081	0.109**
	r_g			1.000	-0.101*	0.493**	-0.461**	0.079	-0.102*	-0.188*	0.276*
NPT	r_p				1.000	-0.207**	-0.124*	-0.196*	0.123*	0.102*	-0.206**
	r_g				1.000	-0.161*	-0.269**	-0.268**	0.145*	0.129*	-0.253**
PL	r_p					1.000	-0.040	0.475**	0.008	-0.058	0.482**
	r_g					1.000	-0.142*	0.601**	0.098	-0.151*	0.596**
PG	r_p						1.000	0.412**	-0.009	0.096	0.375**
	r_g						1.000	0.399**	-0.056	0.139*	0.426**
PW	r_p							1.000	-0.025	0.033	0.833**
	r_g							1.000	0.060	0.026	0.979**
TSW	r_p								1.000	0.007	-0.051
	r_g								1.000	0.008	0.098
AA	r_p									1.000	-0.079*
	r_g									1.000	-0.225**

* : Significant at 5% level; ** : Significant at 1 % level

DFF : Days to 50% flowering	PL : Panicle length	GYP : Grain yield plant^{-1}
DM : Days to maturity	PG : Panicle girth	AA : Alcoholic acidity
PH : Plant height	PW : Panicle weight	
NPT : Number of productive tillers plant^{-1}	TSW : Thousand seed weight	



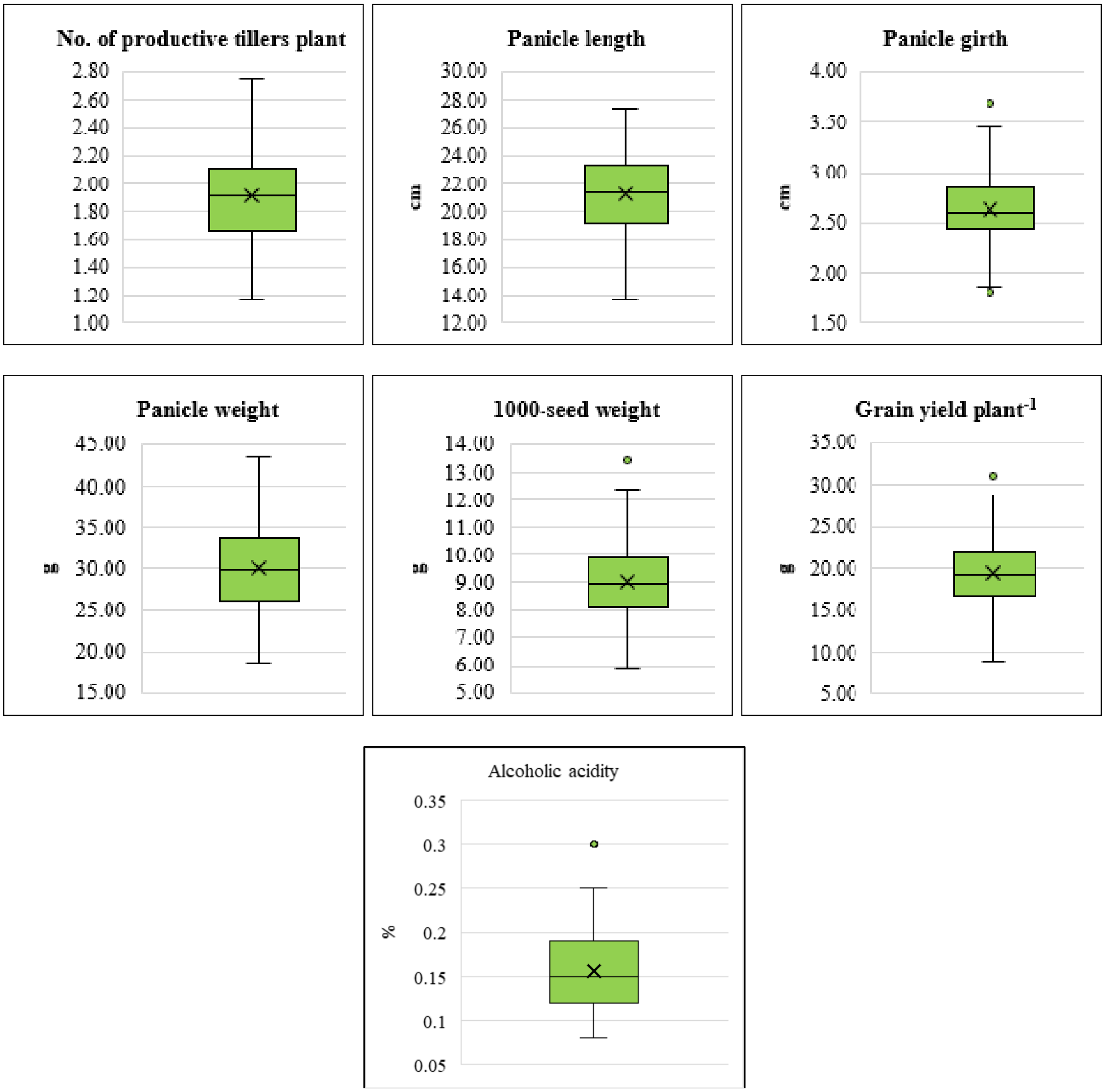


Fig 1: Box plots depicting variation for 10 traits among 100 pearl millet inbreds across the locations (× indicates mean values and indicates median values)

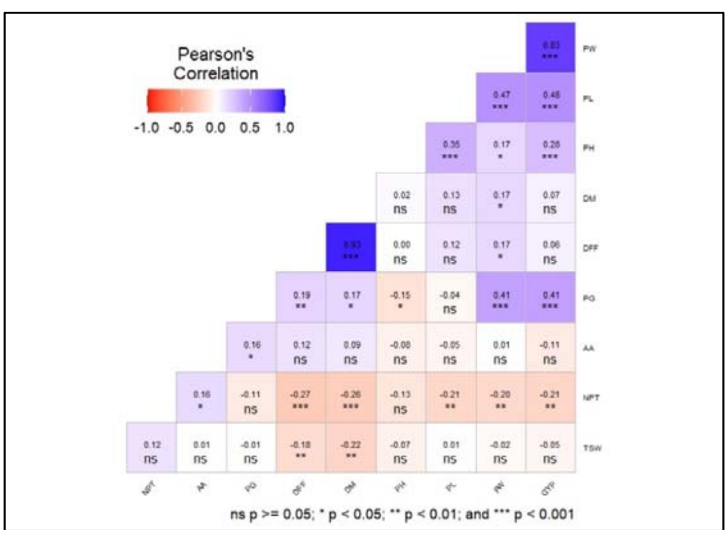


Fig 2: Correlation heatmap showing Pearson correlation coefficients among yield, its component traits and alcoholic acidity in 100 pearl millet inbred lines across the locations

Conclusion
The analysis of variance for individual locations and across the locations showed highly significant differences among inbred lines for all studied traits, indicating substantial variability among them and suggesting ample scope for genetic improvement**.** A considerable variation was observed for most of the traits, as reflected by the range values, which is essential for effective selection and genetic improvement. High estimates of GCV and PCV were observed for alcoholic acidity and moderate estimates for grain yield plant^{-1}, number of productive tillers plant^{-1}, plant height and panicle girth and therefore, selection would be effective for improvement of these traits. High heritability coupled with high genetic advance as percent of mean (GAM) was observed for alcoholic acidity and number of productive tillers plant^{-1} indicating the preponderance of additive gene action. Correlation analysis showed significant positive association of grain yield plant^{-1} with panicle weight followed by panicle length and panicle girth, while it had significant negative association with alcoholic acidity in desirable direction indicating that simultaneous selection of these traits would result in improvement of grain yield plant^{-1} in pearl millet.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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