GENETIC VARIABILITY AND DIVERSITY FOR RANCIDITY AND ASSOCIATED CHARACTERS IN PEARL MILLET (*PENNISETUM GLAUCUM* L.)

ABSTRACT

Pearl millet stands as one of the hardiest warm-season crops globally, offering a rich source of energy, protein, iron, and zinc. The development of high-yielding pearl millet hybrids, characterized by excellent flour quality, is crucial for fulfilling the demands of the food industry. The study focused on assessing yield, rancidity, and associated traits across 49 parental lines, comprising 24 B lines and 25 R lines of pearl millet. Notably, lines 07999R, 04999B, and 08222R were identified as possessing low rancidity levels. Traits such as 1000-seed weight, fat content, lipase, lipoxygenase, peroxidase, and alcoholic acidity on the 5th day of storage exhibited high heritability and significant genetic advance, indicating the influence of additive gene effects. Both genotypic and phenotypic correlations showed a significant positive association between alcoholic acidity on 5th day of storage and the activity of enzymes such as lipase, lipoxygenase and peroxidase. In terms of diversity, Cluster II showcased lines characterized by low alcoholic acidity on 5th day of storage and moderate grain yield per plant.

Keywords: Alcoholic acidity, Rancidity, Genetic variability, Correlations, PCA, Diversity

INTRODUCTION

The escalating global temperatures have underscored the imperative to identify crops resilient to evolving environmental conditions, aiming to address the escalating food demands of an ever-growing global population. Pearl millet (*Pennisetum glaucum* L.), characterized as a C4 crop, demonstrates remarkable photosynthetic efficiency and an innate ability to thrive in adverse conditions such as high temperatures, low rainfall, poor soil fertility, high soil pH, low

soil moisture, and high salinity (Rajeev et al., 2017). These attributes render it well-suited for cultivation under such challenging circumstances[33-36]. Globally, pearl millet ranks 6th in cereal acreage, significantly contributing to food security, particularly in regions across Africa and Asia. It comprises approximately 50% of the total global millet production, standing as the fourth most produced cereal in the world after rice, wheat, and sorghum. Notably, pearl millet is esteemed as an economical and nutrient-rich source of energy, protein, iron, and zinc compared to other cereals and pulses (Rai et al., 2013) with a metabolizable energy content of 2900 Kcal (Singh et al., 2014), starch content ranging between 62-68% (Karamvir, 2015), protein content varying from 7.3% to 13.86% (Anonymous, 2014), and fat content spanning from 4.36% to 7.11% (Karamvir, 2015). Studies have indicated higher antioxidant activity in pearl millet hybrids and varieties compared to other cereals (Kumar et al., 2014), with a reported fiber content of 1.6 g/100 g (Malleshi et al., 1986). Encouraging the substitution of this nutritionally rich millet for staple foods like rice and wheat in India could help address issues such as malnutrition, hidden hunger, obesity, celiac disease, and diabetes (Chandrasekara and Shahidi, 2012).

However, despite its high nutritional value, a major challenge faced by pearl millet is its limited acceptance by the food industry and consumers due to the development of off-flavors characterized by a mousy acidic odor shortly after grinding (Rani et al., 2018). At the household level, large-scale flour preparation is uncommon, particularly in hot climates, due to the rapid onset of rancidity in ungermed flour. The emergence of off-flavors in pearl millet has been attributed to various factors, including the presence of volatile compounds (Thiam et al., 1976), hydrolytic cleavage of lipids (Carnovale and Quaglia, 1973), changes in lipid composition (Lai and Varriano-marston, 1980b), oxidative changes in unsaturated fatty acids (Lai and Varriano-

marston, 1980b), presence of phenolics (Reddy et al., 1986), high peroxidase activity (Banger et al., 1999), and enzymatic changes in C-glycosylflavones (Reddy et al., 1986). Chung and Kumar (2004) suggested that initial levels of lipase and peroxidase activity, as well as phenolic content in grains, may play a significant role in the generation of off-flavors. Additionally, Kumar and Chabra (2008) reported significant positive associations between phenols, C-glycosylflavones, and peroxidase activity with rancidity in pearl millet.

Enzyme lipase catalyzes the conversion of triacylglycerols to di and mono glycerols, glycerols, and fatty acids (Manley and Mayer, 2012), while the abundant presence of linoleic, linolenic, and oleic acids in pearl millet undergo catalysis by lipoxygenase. Primary oxidation leads to the formation of hydroperoxides, subsequently resulting in secondary oxidation products such as aldehydes and ketones responsible for the development of off-odors. A comprehensive understanding of the chemical and biochemical factors contributing to rancidity is currently lacking. Therefore, there is a need to study various morphological and biochemical parameters associated with rancidity development, which can aid in development of hybrids with reduced rancidity, thereby facilitating the efficient utilization of nutrient-rich pearl millet in the food industry. Consequently, this study aimed to identify low-rancidity parental lines in pearl millet for potential use in the development of low-rancidity hybrids suitable for the food processing industry.

MATERIALS AND METHODS

The experimental material comprised of 24 maintainer lines (B lines) and 25 restorer lines (R lines) of pearl millet sourced from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and ICAR-IIMR (Indian Institute of Millets Research). The parental lines were cultivated in medium to light soils within the experimental plots of ICAR-

IIMR, situated in Rajendranagar, Hyderabad (17°19'7"N, 78°24'4"E) during 2017 rainy season. The lines were sown in paired row plots measuring two meters in length, with a row-to-row spacing of 45 cm and plant-to-plant spacing of 15 cm, in two replications following a Randomized Block Design.

The field observations on grain yield, were collected from five randomly selected plants per replication in each genotype and the average was used for statistical analysis. A random sample of 200 seeds of each entry was weighed and multiplied by factor of 5 to obtain 1000 seed weight.

The crude fat (%) was determined using the AOAC method (1999) with slight modifications. Fat from flour was extracted in hexane using the SOCS PLUS system. The lipase activity was estimated following the method outlined by Bier (1955) by hydrolyzing the fat in Olive oil by lipase enzyme into free fatty acids and titrating with sodium hydroxide. The lipoxygenase activity was determined as per the method described by Babitha et al. (2004) with linoleic acid emulsion as the substrate, and the increase in absorbance was measured at 234 nm. Peroxidase activity was estimated based on the method developed by Malik and Singh (1980) and the increase in absorbance was monitored at 430 nm. The alcoholic acidity of pearl millet flour on the 5^{th} day of storage was determined according to IS 12711:1989 Method of Determination of Alcoholic Acidity.

The analysis of variance for the RBD was carried out by following the model given by Panse and Sukhatme (1967). Phenotypic and genotypic variance were estimated as per the procedure given by Lush (1940) and Choudhary and Prasad (1968), while Phenotypic and genotypic coefficient of variance were determined as suggested by Burton (1952). The estimates of heritability and genetic advance as percent mean was estimated as per the procedure of

Hanson et al. (1956) Lush (1949) and Johnson et al. (1955). The correlation between character and fitness was computed for individual environments by means of the correlation coefficients using the formula of Al-Jibouri*et al*. (1958). Trait association was determined by principal component analysis (PCA) (Hatcher 1994) using R version 3.5.1 (R Project for Statistical Computing, (https://www.r-project.org).

RESULTS AND DISCUSSION

Mean Performance of Inbreds: Significant variation was observed among the parental lines for traits such as 1000 grain weight, alcoholic acidity on the 5th day of storage, fat content, and the activity of enzymes lipase, lipoxygenase, and peroxidase, while the variability for grain yield was non-significant as depicted by ANOVA (Table 1). The performance of inbreds for grain yield ranged from 2.17 to 15.19 g per plant, with R13 recording the highest grain yield and 98222B recording the lowest (Table 2). Highest 1000 seed weight was recorded in R38 (13.01 g), followed by 10555R (12.81 g), while 01222B showed the lowest 1000 seed weight (4.64 g). The fat content in the inbred lines ranged from 2.68% to 7.3%, with 10555R and 04888B recording the highest fat content, while the lowest fat content was observed in 13888R. The lines 07999R, 04999B, and 10222B recorded the lowest lipase activity (0.21 units per g tissue), while the highest lipase activity was observed in R43 (0.53 units per g tissue). Significant variation in lipase activity among the parental lines indicates their potential role in off-flavor generation.

The lipoxygenase activity ranged from 41.99 to 279.29 units per gm tissue, with the lowest activity observed in R13 and the highest in 10444B. The peroxidase activity also exhibited wide variation, ranging from 60.32 to 236.8 units per gm tissue, with the lowest activity observed in

11222B and the highest in 06333R. These results indicate substantial variability for grain yield and traits associated with rancidity in pearl millet.

Among the lines tested, alcoholic acidity on the 5th day showed significant variation, ranging from 0.22 to 0.72, with line R22 exhibiting the lowest alcoholic acidity followed by 04999B and the highest observed in 13333R. Increase in alcoholic acidity in pearl millet was reported by several studies. The variation in alcoholic acidity on the 5th day of storage may be attributed to differential activities of enzymes present in pearl millet flour. The fat content in the lines studied ranged from 2.68% to 7.3%, with higher fat content in pearl millet compared to other cereals such as rice, wheat, sorghum, and maize was reported by Saleh et al. (2013) and Joshi et al. (2015). A positive association between fat acidity and fat content was also observed by Preethi and Chugh (2017). Vinutha et al. (2022) identified pearl millet landraces *viz.* Jafrabadi, Chanana Bajra-2, Chadi Bajra and Damodara Bajra as potential genetic material to develop low rancid and they also showed longer and better shelf life.Availability of variation among the inbreds is essential for designing a breeding program. The inbreds studied also showed significant variation for grain yield, alcoholic acidity, and associated traits, indicating their potential use in breeding programs to develop high-yielding, low-rancid pearl millet hybrids for the food industry.In a study with 255 accessions in pearlmillet, lines IP 5695 and IP 19334 were identified as low rancid lines based on lower values of CAV under fresh conditions and all other biochemical parameters under fresh and stored conditions (Bhaghavi et al., 2024).

Genetic Variability: In numerous countries, the increasing emphasis on health has led to a rising demand for nutrient-dense diets. Therefore, besides enhancing food production, addressing the challenges in public health systems has become equally important. Crops such as pearl millet,

known for their high nutrient value and adaptability, hold potential for exploitation in the food industry. However, the main obstacle to its viable use in the food industry is the rancid nature of its flour. Various factors contributing to the development of off-odors in pearl millet include the hydrolytic cleavage of lipids (Cannovale and Quaghi, 1973), initial activities of lipase and peroxidase (Jain, 2013), and the crude fat content of the flour (Preeti et al., 2017). This study aimed to explore the available variability for rancidity and associated traits among 49 parental lines, which could be further utilized in breeding programs to develop low-rancid hybrids for better utilization of nutritionally rich pearl millet in the food industry.

Phenotypic Coefficient of Variation (PCV) exceeded Genotypic Coefficient of Variation (GCV) (Table 3) for all parameters, indicating the influence of the environment on the expression of these traits. The highest GCV and ECV were reported for grain yield per plant, while the difference between PCV and GCV was minimal for fat content, lipase, lipoxygenase, peroxidase, and alcoholic acidity on the 5^{th} day of storage. High heritability coupled with high genetic advance was observed for traits such as 1000 seed weight, fat content, lipase, lipoxygenase, peroxidase, and alcoholic acidity on the 5^{th} day of storage. In contrast, grain yield per plant showed moderate heritability with low genetic advance. The presence of additive gene effects for traits like 1000 seed weight, fat content, lipase, lipoxygenase, peroxidase, and alcoholic acidity on the 5^{th} day of storage indicated the effectiveness of selection for improving these traits, resulting in genetic gains. The difference between GCV and PCV was minimal for fat content, lipase, lipoxygenase, peroxidase, and alcoholic acidity on the 5^{th} day of storage, suggesting a greater role of genetic factors in the expression of these traits and rapid progress from selection for these traits.

Association among the traits: The genotypic correlation coefficient surpasses the phenotypic correlation coefficient for all the traits studied, indicating a robust inherent association among the traits under study (Table 4). The fat content in the seed demonstrates no significant correlation with alcoholic acidity on the 5^{th} day of storage, a finding consistent with Preeti et al. (2015) who also observed a non-significant correlation between fat acidity and fat content. In the current investigation, a significant positive genotypic and phenotypic correlation was observed between alcoholic acidity and the activity of enzymes Lipase, Lipoxygenase, and Peroxidase. Conversely, alcoholic acidity exhibited a significant negative genotypic correlation with grain yield per plant. There was no significant correlation between fat content and the activity of enzymes lipase and peroxidase at both genotypic and phenotypic levels. However, there is a strong association among lipase, lipoxygenase, and peroxidase activity, consistent with the findings of Preeti and Chugh (2017) regarding the strong association between lipoxygenase and peroxidase.Furthermore, Preeti Goyal et al. (2017) proposed that peroxidase activity, fat acidity, and free fatty acids are correlated with each other. The significant positive association of enzymes lipase, lipoxygenase, and peroxidase with alcoholic acidity underscores their pivotal role in the development of off-flavors in pearl millet flour during storage.Aher et al. (2022) also identified that mutation in TAG lipase genes PgTAGLip1 and PgTAGLip2 resulted in lower amounts of free fatty acid accumulation in seeds after milling.

Principal Component Analysis (PCA): PCA analysis yielded seven principal components, as outlined in Table 5. The first three components, with eigenvalues of 1.98, 1.45, and 1.21 respectively, collectively accounted for 66.29% of the total variation. Notably, in these components, grain yield per plant (0.31) and peroxidase activity (0.60) made significant contributions to PC1, while grain yield per plant (0.58) and lipoxygenase activity (0.40) were

more influential in PC2. Additionally, peroxidase activity (0.34), lipoxygenase activity (0.25), and grain yield per plant (0.24) contributed prominently to PC3 (Table 4). Rad et al. (2013) study also revealed that lipoxygenase activity aligned positively with PC1, while alcoholic acidity on the 5^{th} day, lipase, and peroxidase activity showed positive associations with PC2, forming a cohesive group (see fig: 1a). Furthermore, grain yield per plant, 1000 seed weight, and fat content exhibited positive contributions to PC3, forming a distinct grouping. PCA elucidates the contribution of each component to the total variances observed. Notably, the parental lines were distributed across all four quadrants, with 35 lines situated within the first three groups and 14 lines in the fourth group.

CONCLUSION

The successful utilization of pearl millet by the food industry hinges upon the availability of high-yielding varieties with excellent storage characteristics. This investigation has clearly revealed a significant variability in rancidity among the studied pearl millet lines. Among the lines examined, those with low rancidity, specifically 07999R, 04999B, and 08222R hold promise for enhancing the storage quality of pearl millet flour. Consequently, the insights gleaned from this study can be leveraged to pinpoint the lines and genetic factors associated with rancidity in pearl millet, presenting an opportunity to harness these traits for the development of superior hybrids in pearl millet.

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Table 1 Analysis of variance for Grain Yield, Rancidity and other traits in Pearl millet

Character	Mean Sum of Squares	
	Treatments (d.f = 48)	Error (d.f = 48)
Grain yield per plant (g)	23.13	6.72
1000 seed weight (gm)	8.812**	0.350
Fat Content (%)	2.129**	0.137
Lipase (Units/gm tissue)	0.01**	0.00001
Lipoxygenase (Units/gm tissue)	3737.810**	83.408
Peroxidase (Units/gm tissue)	5620.951**	279.221
Alcoholic Acidity	0.0466**	0.0005

Table 2 Grain Yield and rancidity associated characters in pearlmillet inbred lines

S.No	Inbreds	Grain Yield Per plant (g)	1000 seed weight (g)	Fat Content (%)	Lipase (Units/gm tissue)	Lipoxygenase (Units/gm tissue)	Peroxidase (Units/gm tissue)	Alcoholic acidity
1	07999R	6.58	11.14	7.21	0.21	87.65	226	0.33
2	08222R	5.10	8.74	4.27	0.23	141.97	130.1	0.36
3	R 38	11.75	13.01	3.82	0.24	169.28	215.7	0.37
4	R 22	5.94	8.03	5.43	0.23	130.07	135.55	0.22
5	R 13	15.19	8.21	4.86	0.25	41.99	209.9	0.40

6	R 39	6.96	12.4	6.65	0.34	89.28	230.8	0.55
7	08888R	9.04	10.28	6.21	0.3	77.75	120.4	0.49
8	07444R	4.79	8.64	4.2	0.4	151.71	166.55	0.61
9	R 21	7.98	6.41	4.74	0.37	174.05	164.9	0.32
10	12777R	6.40	8.49	5.42	0.34	192.82	190.35	0.57
11	R 14	13.33	12.37	4.48	0.27	159.06	187.65	0.45
12	13333R	3.71	11.41	4.31	0.41	129.78	171.85	0.72
13	R 15	9.79	8.25	6.2	0.35	105.81	204.2	0.59
14	06333R	4.46	8.04	5.68	0.37	114.6	236.7	0.59
15	R 43	11.27	8.94	6.74	0.53	101.17	205.6	0.52
16	R 23	7.37	12.64	4.15	0.29	140.72	230.6	0.49
17	12888R	4.19	9.42	5.21	0.34	199.18	198.4	0.58
18	R 37	14.27	8.27	5.32	0.3	170.67	159.95	0.44
19	10555R	4.44	12.81	7.3	0.25	169.39	169.35	0.42
20	08999R	11.96	6.5	4.26	0.27	152.42	194.05	0.44
21	06111R	5.60	9.45	4.67	0.27	176.77	236.8	0.44
22	R 17	13.98	11.72	2.88	0.27	103.61	225.7	0.46
23	R 30	7.00	7.69	4.9	0.32	118.3	216.4	0.54
24	13888R	5.44	8.5	2.68	0.36	120.78	195.55	0.61
25	R 40	13.25	9.84	4.61	0.39	110.07	210.7	0.49
26	97111B	3.94	11.05	5.48	0.25	78.7	91.5	0.40
27	99222B	4.15	5.74	4.25	0.23	213.91	147.5	0.36
28	01222B	5.58	4.64	5.21	0.3	189.28	131.9	0.50
29	02666B	4.00	5.7	4.88	0.27	153.68	137.3	0.45
30	03999B	5.00	10.15	4.41	0.23	164.83	104.8	0.36
31	04888B	4.48	11.89	7.3	0.3	126.97	90.6	0.50
32	03333B	3.27	9.97	3.47	0.42	171.62	105.25	0.61
33	02555B	13.39	9.79	5.5	0.23	103.16	111.2	0.36
34	93333B	11.17	8.33	3.13	0.23	129.48	107.89	0.46
35	07999B	7.58	11.88	5.3	0.3	151.82	65.48	0.58
36	98222B	2.17	10.98	4.63	0.36	172.19	108.1	0.41
37	04999B	6.27	6.89	4.68	0.21	145.45	84.81	0.30
38	10222B	6.10	10.38	7.21	0.21	181.16	104.48	0.61
39	11222B	4.14	10.63	4.27	0.23	111.43	60.32	0.64
40	08666B	7.62	8.7	4.63	0.32	113.45	80.07	0.34
41	88004B	5.25	11.99	5.83	0.34	131.99	104.74	0.62
42	92777B	6.85	11.48	4.98	0.23	154.27	139.85	0.49
43	94444B	6.19	12.16	4.12	0.32	236.18	208.8	0.42

44	94555B	2.73	11.81	4.78	0.3	175.03	111.79	0.40
45	03666B	9.19	10.38	5.69	0.34	138.54	80.93	0.44
46	05444B	6.92	7.78	4.69	0.36	137.11	91.84	0.42
47	10444B	5.54	10.87	4.72	0.41	279.29	185	0.64
48	10999B	4.15	6.99	5.17	0.36	185.69	132.8	0.52
49	05888B	5.44	8.28	4.73	0.48	187.8	106.05	0.54
	Mean	**7.16**	**9.58**	**4.96**	**0.33**	**146.16**	**153.61**	**0.48**
	Range	**2.17 - 15.19**	**4.64-13.01**	**2.68-7.3**	0.21-0.48	**41.99-279.29**	**60.32-236.8**	**0.22 – 0.72**
	CD	**5.22**	**1.194**	**0.746**	**0.002**	**18.408**	**33.702**	**0.02**
	SE (m)	**1.83**	**0.419**	**0.261**	**0.001**	**6.454**	**11.816**	**0.007**
	CV	**36.20**	**6.177**	**7.455**	**0.317**	**6.245**	**10.878**	**2.05**

Table: 3 Estimates of genetic variability, heritability and genetic advance as percent mean for yield and rancidity associated characters in Pearlmillet

GeneticParameter	Range	Grand Mean	PCV (%)	GCV (%)	ECV (%)	h^2bs (%)	GAPM
1000seedweight(g)	4.64 –13.01	9.584	22.333	21.462	6.177	92.35	42.487
Grainyieldperplant(g)	2.17 –15.19	7.16	71.88	62.10	36.20	55.70	16.02

Fat Content (%)	2.68 –n7.3	5.40	21.458	20.122	7.453	87.93	42.123
Lipase (Units/gm tissue)	0.21 – 0.48	0.33	22.496	22.494	0.000	99.98	46.529
Lipoxygenase (Units/gm tissue)	41.99 – 279.29	146.16	29.899	29.239	6.248	95.63	58.914
Peroxidase (Units/gm tissue)	60.32 – 236.8	153.61	35.360	33.645	10.870	95.15	69.308
Alcoholic Acidity	0.22 – 0.72	0.48	22.252	22.357	0.09	97.93	45.660

Table4: Genotypic and phenotypic correlation coefficient for yield and rancidity associated traits in Pearl millet

Characters		Grain Yield Per plant (g)	1000 seed weight (g)	Fat Content (%)	Lipase (Units/gm tissue)	Lipoxygenase (Units/gm tissue)	Peroxidase (Units/ gm tissue)	Alcoholic acidity
Grain Yield Per plant (g)	r_g	1.00	0.035^{NS}	-0.080^{NS}	-0.173^{NS}	-0.439^{**}	0.224^{*}	-0.246^{*}
	r_p	1.00	-0.010^{NS}	-0.107^{NS}	-0.129^{NS}	-0.323^{**}	0.022^{NS}	-0.117^{NS}
1000 seed weight (g)	r_g		1.00	0.139^{NS}	0.060^{NS}	-0.083^{NS}	0.273^{**}	0.164^{NS}
	r_p		1.00	0.128^{NS}	0.058^{NS}	-0.068^{NS}	0.071^{NS}	0.039^{NS}
Fat Content (%)	r_g			1.00	0.032^{NS}	-0.220^{*}	-0.034^{NS}	0.084^{NS}
	r_p			1.00	0.030^{NS}	-0.192^{NS}	-0.017^{NS}	0.029^{NS}
Lipase (Units/gm tissue)	r_g				1.00	0.201^{*}	0.838^{**}	0.635^{**}
	r_p				1.00	0.197^{NS}	0.267^{**}	0.993^{**}
Lipoxygenase (Units/gm tissue)	r_g					1.00	0.968^{**}	0.204^{*}
	r_p					1.00	0.263^{**}	0.199^{*}
Peroxidase (Units/ gm tissue)	r_g						1.00	0.913^{**}
	r_p						1.00	0.246^{*}
Alcoholic acidity	r_g							1.00
	r_p							1.00

*significant at 0.05 level of probability, **significant at 0.01 level of probability

Fig. 1a Principal component analysis for individual traits

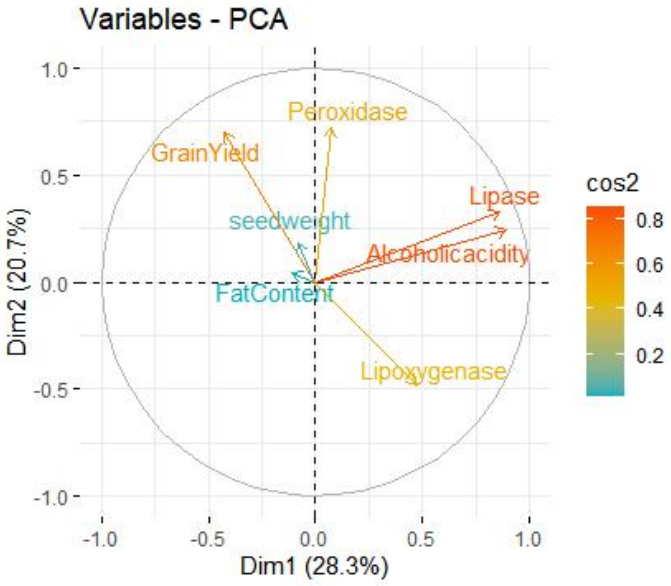

Fig. 1b Principal component analysis for individuals

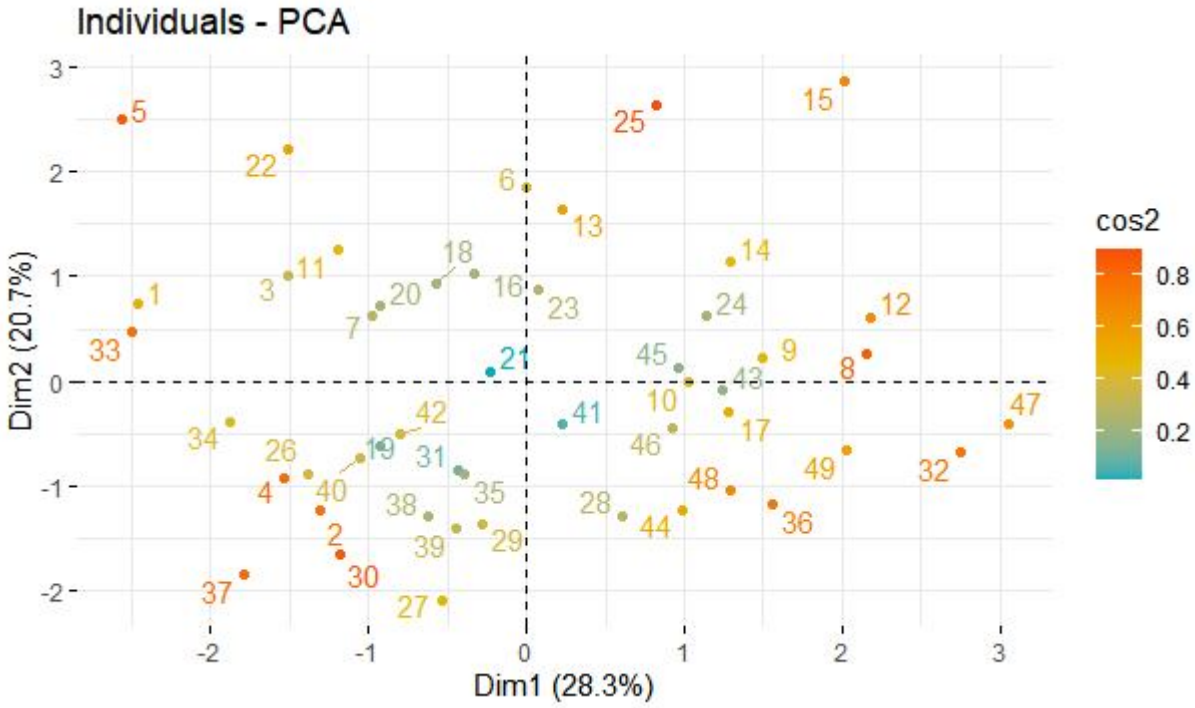

Table 5 Eigen values and percent variance contribution by each PCA

PCA	Eigen Value	Variation %	Cumulative Variance %
PC1	1.98	28.28	28.27
PC2	1.45	20.68	48.96
PC3	1.21	17.33	66.29
PC4	0.94	13.43	79.72
PC5	0.75	10.74	90.46
PC6	0.48	6.93	97.39
PC7	0.18	2.61	100

Table 6 PCA loadings for individual traits

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Grain yield per plant	0.31	0.58	0.24	0.00	0.15	0.69	0.00
1000 seed weight	0.00	0.15	-0.55	-0.72	0.37	0.00	-0.11
Fat content	0.00	0.00	-0.73	0.29	-0.55	0.27	0.00
Lipase	-0.61	0.27	-0.21	0.10	0.00	0.00	-0.69
Lipoxygenase	-0.34	-0.40	0.25	-0.47	-0.39	0.53	0.00
Peroxidase	0.60	0.18	-0.34	-0.58	-0.39	0.00	0.00
Alcoholic acidity	-0.63	0.19	-0.12	0.00	0.19	0.00	0.71