Genetic Diversity Studies in Indigenous Pearl Millet [*Pennisetum glauccum* (L.) R. Br.] Accessions Based on Biometrical and Nutritional Quality Traits

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The aim of this research was to study genetic divergence among the pearl millet [*Pennisetum glauccum* (L.) R. Br.] accessions obtained from Millet Breeding Station, Coimbatore and clustering them into homogenous groups based on yield components and nutritional quality traits for the hybridization programme. Genetic divergence analysis was done based on multivariate analysis using Mahalanobis's D^2 statistics. The experimental material consists of 61 elite germplasm lines grouped into eight different clusters based on yield components, nutritional and anti-nutritional traits. The maximum divergence with high mean performance was observed between clusters II, V and VII. Hence, hybridization between the genotypes of these clusters may exhibit high heterosis as well as high level of production and quality. Zinc content contributed maximum to the genetic divergence followed by phytate phosphorus and crude fat content. The result Indicate that, presence of ample variation for grain zinc in pearl millet germplasm can be exploited for further improvement of pearl millet cultivars with respect to grain zinc content. The low contribution to genetic divergence by other characters may be due to precedent domestication and selection towards uniformity for yield characters. The presence of significant genetic variability among the evaluated germplasm accessions suggests that, variability can be exploited for improvement of grain nutritional quality and yield traits through hybridization of genotypes from different clusters and subsequent selection from the segregating generations.

Key Words: Cluster distance, Genetic divergence, Germplasm accessions

Introduction

Pearl millet [*Pennisetum glauccum* (L.) R. Br.] is an important food crop and is vital source of energy for the millions of poor inhabiting the semi-arid tropics. This crop occupies 10 m ha in India which shares one third of world production area. The crop is cultivated for grain and fodder purpose in the semi-arid regions of Africa and Indian subcontinent (Devos *et al.,* 2006). However, in United States (US) and Europe, pearl millet is grown as a fodder and feed crop for livestock. The quest for increasing yield is though, a primary concern for an increasing world population which has achieved self-sufficiency in food production quantitatively (green revolution), but not in quality. Hence, priority of breeders may be to breed for quality with higher amount of micronutrients especially iron (Fe) and zinc (Zn) in edible part (grains), which is most frequent deficient minerals in cereal based human diets and globally estimated that five million people are deficient in both the elements. As pearl millet is highly cross pollinated, genetic variability in the species is well distributed both within and among cultivars. Assessment of genetic variability and identification of superior genotypes are prerequisite for crop-breeding programme. Pearl millet produces nutritious grains that are a rich source of protein, calcium, phosphorus, iron and zinc compared to other major cereal crops (Devos *et al.,* 2006). Millets typically contain higher quantities of essential amino acids especially methionine, cysteine and fat content than maize, rice, wheat and sorghum (Obilana and Manyasa, 2002). Being drought-tolerant, higher protein concentration and protein quality compared to other cereals, scientists hope to increase its use as feed grain for both humans and animals alike. Recently, a large variation for grain Fe (30.1 to 75.7 mg kg^{-1}) and Zn (24.5 to 64.8 mg kg^{-1}) content in pearl millet germplasm accessions, breeding lines, improved populations, commercial open-pollinated varieties (OPVs) and hybrid parents have been reported by Velu *et al*., 2007. Similarly, variability for grain Fe (46.9 to 85.6 mg kg^{-1}) and Zn (36.4 to 69.9 mg kg^{-1}) was reported in commercial and pipeline hybrids developed in India (Velu *et al*., 2008). Till recently, only few genetic studies have been carried out for micronutrients especially for grain Fe and Zn (Jambunathan and Subramanian, 1988). Genetic resources of pearl millet are untapped that need attempt to improve protein content in harvested varieties for significant yield improvements (Rai *et al*., 1999).

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In spite of, having superior grain quality, millet contains some anti-nutritional factors such as phytic acid, which reduce bioavailability of nutrients. At present anti-nutritional factors are low in pearl millet but in future, their level need to be monitored to keep them at lower levels (Govindaraj, 2006). Large number of the population suffers from chronic malnutrition where pearl millet is used as food crop, (Khush, 2008). Thus, improvement in nutritive quality of grains along with the sustained yield may prove useful for alleviating malnutrition. Aforesaid facts suggest that, pearl millet has all the potential, and present study was conducted to determine diversity and superior lines for use as parent in hybrid development

Materials and Methods

Sixty-one pearl millet genotypes were selected based on earlier studies on yield and its componential traits for the present study at Millet Breeding Station, Tamil Nadu Agricultural University (TNAU), Coimbatore, to study and evaluate genetic divergence by D^2 statistics. They are currently being used in breeding programs at the same centre/or at the breeding programs at state agricultural universities and national research centre in India. Seeds of these accessions were maintained by the Millet Breeding Station (now Department of Plant Genetic Resources (Table 1). The field experiment was carried out during 2006 under the prevailing environmental conditions at TNAU, Coimbatore. All the entries were sown manually and the plot size of 4 m and row-to-row and plant-to-plant distance was 60 and 15 cm, respectively, in a Randomized Complete Block (RCBD) Design with three replications. The crop was grown under uniform conditions to minimize environmental variability to the maximum possible extent. The data were recorded on five randomly selected plants in each plot at each replication for eight morphological characters (Table 2).

Table 1. List of genotypes used in the study (materials source: millet breeding station, TNAU, Coimbatore

Sl. No.	Genotypes	Sl. No.	Genotypes
1.	PT1796	31.	PT 4664
2.	PT 2198	32.	PT 4760
3.	PT 2243	33.	PT 4896
4.	PT 2582	34.	PT 4976
5.	PT 2610	35.	PT 5005
6.	PT 2659	36.	PT 5010/2
7.	PT 2835/1	37.	PT 5021
8.	PT 3311	38.	PT 5072
9.	PT 3397	39.	PT 5077
10.	PT 3488	40.	PT 5099
11.	PT 3559	41.	PT 5118
12.	PT 3657	42.	PT 5179
13.	PT 3687	43.	PT 5188 (Y)
14.	PT 3718	44.	PT 5241
15.	PT 3755	45.	PT 5541
16.	PT 3758	46.	PT 5547
17.	PT 3764	47.	PT 5552
18.	PT 3987	48.	PT 5554
19.	PT 4060	49.	PT 5564
20.	PT 4219	50.	PT 5604
21.	PT 4266/2	51.	PT 5605
22.	PT 4377	52.	PT 5625
23.	PT 4440	53.	PT 5722
24.	PT 4464	54.	PT 5744
25.	PT 4470	55.	PT 5765
26.	PT 4508	56.	PT 5843
27.	PT 4551	57.	PT 5856
28.	PT 4572	58.	PT 5864
29.	PT 4591	59.	PT 5913
30.	PT 4619	60.	PT 5914
		61.	PT 5939

PT – *Pennisetum typhoides* (old botanical name)

Table 2. Analysis of variance for biometrical and mineral quality characters in indigenous pearl millet accessions

Source of variation	df	Mean Squares												
		Day to 50% flowering	Plant height (cm)	No. of productive tillers	Panicle length (cm)	Panicle girth (cm)	Days to maturity	100-grain weight (g)	Grain yield/ plant (g)	Crude protein (%)	Crude fat (%)	Phytate phosphorus (mg)	Phosphorus (mg)	Calcium (mg)	Iron (mg)	Zinc (µg)
Replication	2	0.051	2.685	0.026	0.292	0.077	0.234	0.000	2.602	0.735	0.025	0.374	264.6	0.265	0.011	0.2461
Genotypes	60	12.994**	486.21**	0.30**	8.95**	0.451**	5.27**	0.003**	73.78**	5.11**	2.43**	1438.21**	1690.62**	40.39**	2.88**	1271.45**
Error	60	0.517	20.340	0.009	0.430	0.037	0.192	0.001	1.460	0.243	0.008	0.855	146.455	0.390	0.007	0.2739

** Significant at $P < 0.01$ level

After thrashing, cleaning and weighing, the grains from each entry were dried in hot air oven at 60^0 C for 6h. The grains were then ground in Willey mill separately, and the powder was stored in properly labeled butter paper cover for further analysis. The crude protein content was estimated using method by Humphries (1956). The crude fat determined by Soxhlet apparatus with petroleum ether (AOAC, 1960). Phytic acid estimation was done using the method of Wheeler and Fernel (1971), estimation of calcium was based on versenate titration method as suggested by Jackson (1973). Phosphorous from the grain sample was determined as per Vanadomolybdo phosphoric yellow color method (Piper, 1966). The Fe and Zn estimation were done by using Atomic Absorption (Jackson, 1973). Protein and fat estimation carried out at the Department of Forage, Centre for Plant Breeding and Genetics, TNAU. Determination of calcium, phytate phosphorus and phosphorus were carried out at Department of Soil Science and Agricultural Chemistry, TNAU. The iron and zinc content estimated at Department of Environmental Science, TNAU. The statistical analysis was done for ANOVA by using GenStat 12th edition Statistical software (VSN International Ltd, 2009). Genetic divergence analysis was computed based on multivariate analysis using Mahalanobis's D^2 statistic (Mahalanobis, 1936) using NTSYS statistical software (Rohlf, 1996).

Results and Discussion

Analysis of variance revealed significant differences between the genotypes for all the characters studied, revealing the existence of substantial amount of variation among the genotypes (Table 2). The Mahalanobis D^2 statistic has been found to be favourable for estimating genetic divergence between genotypes for selection in the breeding programme. The success in obtaining highly heterotic hybrids and creating greater variability for efficient selection of useful recombinants in breeding programmes depend on the degree of divergence between the parents chosen (Murty and Tiwari, 1967). For exploiting heterosis as a means of increasing production, it is necessary to utilize parents with maximum genetic divergence. The more diverse the parents more are the chances of pronounced heterotic effects and increased spectrum of variability in the segregating generations.

In the present study, Wilks criterion was used for simultaneous test of significance of the differences in the mean value of character. Highly significant difference was observed among genotypes for all the characters,

suggesting the existence of considerable divergence in the given genetic material and justified the need to estimate squared distance values for the genotype combinations using these characters. Diversity based on either quality characters or mineral content is not given much importance in the past breeding track whilst the aim of the breeding programme is mainly focused on yield improvement. Hence, diversity based on quantitative characters is preferable than diversity based on quality characters.

Based on D^2 value estimates of genetic divergence, the 61 pearl millet accessions were grouped into eight distinct clusters (Fig. 1).The cluster analysis suggested the resolution of sixty one genotypes into eight distinct clusters following Tocher's method of clustering, indicating wide diversity in the experimental material for majority of the characters studied including nutritional characters. Cluster I consisted of a maximum of 31 genotypes (51 %), cluster III consisted 15 accessions (25%) and II consisted six accessions (10%) of the genetic materials used in the study (Fig 1). Remaining clusters (V, VI, VII, and VIII) were contributing only one genotype each. The uneven distribution of genotypes to different clusters and most genotypes falling into few clusters suggested that, indigenous germplasm collected from the same geographic area were not necessarily closely related and different regions did not necessarily have different genetic background and being spread in the country. Similar results were reported by various researchers previously (Garg and Gautam, 1988; Walia and Garg, 1996; Singh *et al*., 2003; Prabhu *et al*., 2005). Cluster IV comprising of five genotypes had the highest intra cluster distance (94.26) indicating the high divergence among the genotypes of the cluster. While considering the inter cluster distances, it was found that minimum inter cluster distance (81.17) was noticed between cluster I and V which explain that the genotypes in these clusters would have been evolved by similar evolutionary procedure. Thus, crossing of genotypes from these two clusters may not produce a high amount of heterotic expression in the F_1s and broad-spectrum of variability in segregating (F_2) populations (Gashaw *et al.,* 2007). Maximum inter cluster distance (299.78) was noticed between cluster II and V (Table 3). Lines for hybridization can be selected on the basis of large inter-cluster distance to generate useful recombinants in the segregating populations. Increasing parental distance implies a greater number of constraining alleles at the desired loci, and then to the extent that these loci recombine in the F_2 and F_3 generations following a cross of distantly related parents, the greater will be the opportunities for successful selection for any character of yield interest (Ghaderi *et al*., 1984) for instance, the present study showed considerable variability for panicle length which was depicted in the figure 2. (Data were not shown).

Cluster mean performance of grain yield and other important contributing characters such as, number of productive tillers (4.35), panicle length (27.60 cm), panicle girth (6.85 cm), 100 grain weight (1.07 g), grain yield/plant (77.22 g), crude protein (13.22), and phosphorus (361.80 mg) was highest for cluster VII and moderately high for cluster II with high iron and zinc, indicated that, inbreds from this genetic cluster will serve as potential parents for improvement of grain Fe and Zn content, which is most prevalent deficiency in human beings where, there is inherent soil deficiency. Cluster V had high mean value for calcium, early maturity, low fat content and considerably low phytate phosphorus (Table 4). It is important to note that, early flowering and early maturity makes the cultivars more suitable by enabling them escape from terminal drought (end of the season), in this regard cluster V and VI had the early maturity and early flowering genotypes respectively, suggests crossing between individual from these clusters will produce hybrids suitable for cultivation in drought prone environments of western and northern parts of India. Keeping this in mind, it appeared that crosses between genotypes belonging to clusters II, V, and VII in all possible combination would not only exhibit high heterosis but also increase the level of production and nutritional quality where pearl millet plays an important role in human food and industrial sector, such as poultry feed and pig feed with high nutritious values. Hence, it is essential to improve the quality of pearl millet. The outstanding *per se* performance by the each genotype from the selected clusters may serve as potential genotypes for future breeding programme with respect to traits concerned (Table 5).

In addition to general features of variation and divergence, this study also provides the information on the potent characters that contributes to the divergence between the genotypes. It was found that the maximum contribution to the genetic divergence was accounted by zinc content (65.41%) followed by phytate phosphorus (23.17%) (Table 4). The low contribution to genetic divergence by other characters may be due to the fact that selection towards uniformity in these characters could have caused an eroding effect on genetic diversity (Das and

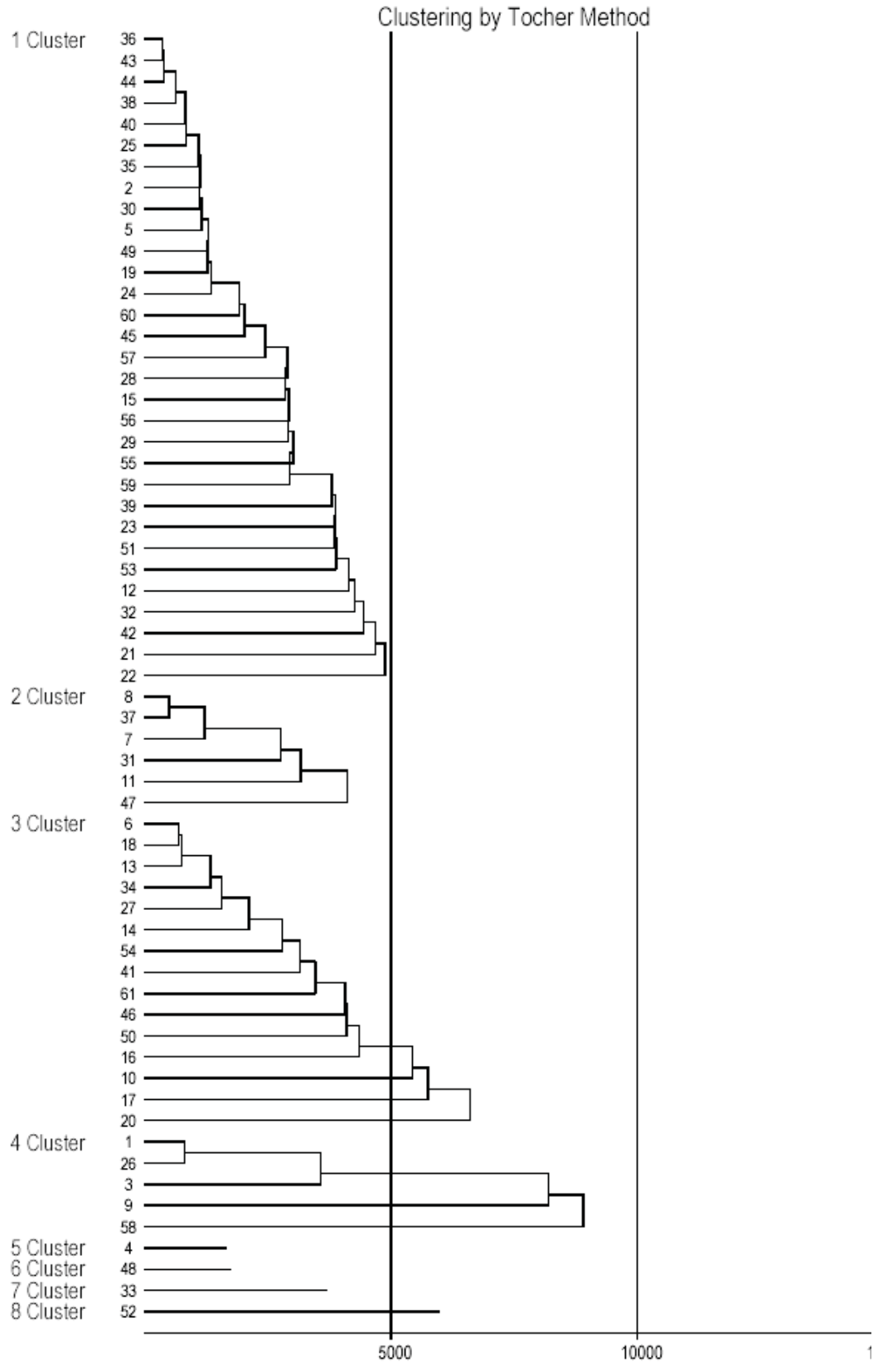

Fig. 1. Clustering for 61 genotypes in pearl millet based on fifteen characters

Table 3. Average intra- (in bold) and inter-cluster D^2 distances in indigenous pearl millet accessions

Cluster No.	I	II	II	IV	V	VI	VII	VIII
I	**58.10**	276.17	139.83	238.14	81.17	212.90	87.49	144.52
II		**61.03**	157.94	171.89	299.78	94.79	240.42	256.16
III			**68.57**	160.17	165.08	103.90	117.79	151.41
IV				**94.26**	225.12	118.08	230.41	148.54
V					**0.00**	225.48	129.56	102.63
VI						**0.00**	185.12	179.45
VII							**0.00**	175.43
VIII								**0.00**

Table 4. Cluster mean values for biometrical and nutritional quality characters in indigenous pearl millet accessions

Cluster No.	Day to 50% flowering	Plant height (cm)	Number of productive tillers	Panicle length (cm)	Panicle girth (cm)	Days to maturity	Crude protein (%)	Crude fat (%)	Phytate phosphorus (mg)	Phosphorus (mg)	Calcium (mg)	Iron (mg)	Zinc (µg)	100 grain weight (g)	Grain yield per plant (g)
I	48.69	192.63	3.73	23.29	6.25	85.39	12.43	3.94	213.73	345.6	36.69	2.43	117.26	1.01	61.61
II	48.67	194.33	3.73	22.54	6.09	84.94	12.31	4.02	225.41	353.6	37.74	5.22	**186.17**	0.99	62.28
III	49.65	198.67	3.80	23.11	6.07	85.02	11.98	4.04	216.46	343.2	33.29	3.76	149.82	1.01	62.36
IV	49.10	202.00	3.52	20.89	6.42	84.55	12.43	3.77	141.51	289.2	34.06	4.43	167.42	0.99	60.48
V	**53.50**	211.5	3.80	**19.90**	6.13	**82.91**	11.99	**3.15**	169.95	306.0	**42.85**	2.46	**113.75**	**0.96**	52.87
VI	**46.50**	**174.5**	**3.35**	23.12	6.29	85.00	**10.28**	3.95	200.37	351.6	41.12	**5.46**	169.01	1.01	52.29
VII	47.50	198.00	**4.35**	**27.60**	**6.85**	**87.78**	**13.22**	**6.90**	**229.53**	**361.8**	41.65	4.65	131.66	**1.07**	**77.22**
VIII	**53.50**	**203.50**	**3.35**	22.60	**5.98**	85.24	10.57	3.37	**138.95**	**269.9**	**31.56**	**2.07**	131.50	1.01	**52.25**
% contribution to divergence	0.05	0.05	–	0.11	–	–	0.71	5.96	23.17	–	0.66	3.66	65.41	0.11	0.11

The bold numbers are the highest and lowest mean values for each character

Table 5. Outstanding genotypes based on yield and nutritional characters in the selected genetic clusters

Clusters No. (1)	Characters (2)	Genotypes (3)
II	Iron and Zinc	High: PT 1796, PT 2835/1, PT 4508, PT 4664, PT 5021, PT 5552
	Days to 50% flowering	Late: PT 2582, PT 2835/1, PT 4551, PT 4572, PT 5625, PT 5939
	Panicle length	Low: PT 1796, PT 2243, PT 2582
	Days to maturity	Early: PT 5604, PT 5864
V	100-grain weight	Low: PT 4470, PT 5552
	Crude fat	Low: PT 5099, PT 2659, PT 3718, PT 4440, PT 5765, PT 5864, PT 5188 (Y)
	Calcium	High: PT 2835/1, PT 4664, PT 5843
	Zinc	Low: PT 5241, PT 5179, PT 5188 (Y), PT 4266/2, PT 4464
	Days to 50% flowering	Early: PT 3687, PT 3311, PT 3657, PT 3764, PT 5765
	Plant height	Dwarf: PT 5072, PT 5021, PT 4060, PT 5099, PT 5118
VI	Number of productive tillers	Low: PT 3657, PT 3488, PT 2243
	Crude protein	Low: PT 5604, PT 3488, PT 3764, PT 4377, PT 4591, PT 5021
	Number of productive tillers	High: PT 5939, PT 4760, PT 4896
	Panicle length	High: PT 5547, PT 4896, PT 5913, PT 5939
	Panicle girth	High: PT 5914, PT 5547, PT 2243, PT 5605, PT 5552, PT 5541
VII	Days to maturity	Late: PT 4508, PT 4551, PT 4266/2, PT 4572, PT 4377, PT 4664, PT 4760
	100-grain weight	High: PT 5856, PT 5744, PT 5179, PT 4896, PT 4760, PT 5722
	Grain yield/plant	High: PT 5939, PT 4896, PT 5856, PT 5914, PT 5744, PT 5179, PT 4760, PT 3559
	Crude protein	High: PT 5118, PT 3718, PT 3657, PT 4508, PT 5564, PT 5843, PT 5914
	Crude fat	High: PT 3758, PT 4760, PT 4896, PT 5179, PT 5744, PT 5856
	Phytate phosphorus and Phosphorus	High: PT 4377, PT 4219, PT 4664
	Days to 50% flowering	Late: PT 2582, PT 2835/1, PT 4551, PT 4572, PT 5625, PT 5939
	Plant height	Tall: PT 5939, PT 4760, PT 4664, PT 2582, PT 3559, PT 5010/2, PT 5077
	Number of productive tillers	Low: PT 3657, PT 3488, PT 2243
	Panicle girth	Low: PT 3764, PT 5021, PT 3987, PT 5564
VIII	Grain yield/plant	Low: PT 3755, PT 2582, PT 3488, PT 4060, PT 4619, PT 5554, PT 5625
	Phytate Phosphorus	Low: PT 1796, PT 2243, PT 4508, PT 4591, PT 5625, PT 5864, PT 2582, PT 3397, PT 5010/2, PT 5072, PT 5547, PT 5914, PT 5939
	Phosphorus	Low: PT 5547, PT 5625, PT 2610, PT 3397
	Calcium	Low: PT 5552, PT 5547, PT 1796, PT 3488, PT 5241, PT 5564, PT 5604
	Iron	Low: PT 4464, PT 5010/2 PT 5072, PT 5188 (Y) PT 5241 PT 5541

Borthakur, 1973) which showed that genetic variability was reduced in the course of domestication and selection. There is possibility of operation of a similar phenomenon on genotypes of certain clusters showing less contribution towards the genetic divergence.

From this study, genotypes in clusters II, V and VII possess desirable combinations of traits and thus, the genotypes of these three clusters hold great promise as parents to obtain promising heterotic expression in F_{1s} and may create considerable variability in the segregating populations. The wealth of information will help to select the superior indigenous accession for productivity with acceptable nutritional qualities, which are not only exploited for the current agricultural advances, but can

also be used to generate the genetic resources, that can be employed in hybridization programme for nutritional improvement in human as well as animals.

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