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Genetic variations for grain mineral content in pearl millet germplasm and its breeding implications and future outlook

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Abstract

Germplasm are recognized as valuable genetic resources for improvement of any traits. Sixty one germplasm of pearl millet of diverse for one or many characters were selected and evaluated in completely randomized block design at millet breeding station, Tamil Nadu Agricultural University, Coimbatore to study and evaluate the extent of variation and breeding prospect for nutritional quality characters. A wide range of variability existed for all the traits studied indicating the presence of significant variability among the tested genotypes. In present study, the relationship between grain iron and zinc content was significant (r^2 = 0.77; P <0.001) and simple selection can enhance the levels simultaneously for the both elements. On the negative side, total phosphorus significantly associated with phyate (r^2 = 0.64; P <0.001), indicates the selection for low phytate will not operates but moderate to high phosphorus with considerable level of phytate can be achieved by repeated selection. Selection for high yield and improved mineral content for cereals can be achieved in near future and such varieties could significantly improve wellbeing of both the plants and the people who rely upon them.

Key words: Malnutrition, iron, zinc, pearl millet, selection

Introduction

Cereal foods are naturally nutritious in fact next to fruits and vegetables (Jones, 1999). Among cereals, millets are hardiest food crop grow in harsh environments where other crops do not grow well. Over 50 per cent pearl millet grain production in Asia is utilized for food purpose and the 20 per cent is used for feed (Velu *et al.*, 2007). The nutritional value of this crop, especially protein, fat and mineral contents (iron and zinc) is comparable or even superior to those of other cereal food grains (Aykroyd, 1956) Improvements in production, availability, storage, utilization and consumption of these food crops will significantly contribute to the household food and nutrition security of the inhabitants of semi arid tropic of the world. Generally, nutritional factors are difficult for the breeder to work with, because of both an analytical procedure is needed, and because rapid analytical techniques for small quantities of grain are seldom available. However, modern technology is steadily removing the analytical difficulty. The genetic information about these nutritional qualities in pearl millet is essential to develop the pearl millet strain with high quality coupled with high grain yield. The sizeable studies on grain quality characters for pearl millet were available in the past nevertheless it has not been deeply studied and reviewed. Goswami *et al.* (1969a, 1969b, 1970a, and 1970b) have reported protein content of a large number of pearl millet varieties of Indian, African and American origins. The range of protein was between 9.82 and 15.25 per cent (Chandna and Matta (1990). Gotmare and Govila (1999) showed wide variability of proteins from 10.30 to 18.75 per cent in grey and from 12.3 to 19.4 per cent in white grained inbred lines and it had high heritability. (Mangal *et al*, 2004).

With esteem to mineral content, studies started by Goswami *et al.* (1970 a) and subsequently, a wide range of mineral content in pearl millet was reported by Varriano-Marston and Hoseney (1980). The total phosphorus ranged from 263.85 to 342.93 mg kg^{-1} (Arulselvi, 2004). Unfortunately, most of the phosphorus in cereal grains is stored in the form of phytic acid. A significant positive correlation between total phosphorus and phytate-phosphorus in pearl millet grains was observed by Sharma and Kapoor (2000). On the other side, breeding for low level of phytic acid is likely to reduce the concentration of protein (Raboy *et al.,* 1991). A large genetic variability among the genotypes was reported for grain Fe (30.1to 75.7 mg kg^{-1}) and Zn content (24.5 to 64.8 mg kg^{-1}) (Velu *et al.*, 2007) and wider genetic variability for mineral content, which showed the spread of variation for these characters in pearl millet and also recorded high heritability and genetic advance. There is a clear evidence of poor nutrition among Indians; they suffer from severe malnutrition which is caused by consumption of mono-cereal foods (deficient in micronutrients) and improper balanced diet. Nowadays consumer believes grains are the most healthy food overall and it has to be proven an excellent carrier for nutritional substances. Therefore, the primary objective of the present study is to screen available germplasm resources for variability in better grain nutrition quality and breeding strategies for their genetic improvement.

Materials and Methods

Field experiments: Sixty pearl millet (*Pennisetum glaucum* (L.) R. Br.) genotypes were obtained from the Department of Millets, Tamil Nadu Agricultural University (TNAU), Coimbatore and were used as test materials for the present investigation. These lines were chosen based on their variability for grain yield and grain size during earlier selections. The experiment was laid in a randomized blocks design with two replications. Each genotype was represented by a two rows of 4 m length with a spacing of 60 cm between rows and 15 cm between plants. All recommended agronomic practices were adopted. At the physiological maturity panicles were harvested in each entries and careful manual threshing was done.

Grain minerals analyses: After threshing, cleaning and weighing, the grain samples from each entry were dried in hot air oven at 60^0 C for 6 hours. The grains were then ground in

Willey mill separately, and labeled properly and stored in butter paper cover for further analysis. For each element analyses 100 gram of homogenized sample were taken. The crude protein content was estimated using method suggested by Humphries (1956) and the crude fat was determined by soxhlet apparatus with petroleum ethane (A.O.A.C., 1960) at the Department of Forage crops, Centre for Plant Breeding and Genetics, Coimbatore. Phytic acid estimation was done based on the method of Wheeler and Fernel (1971), calcium was estimated in pearl millet grain sample following versenate titration method as suggested by Jackson (1973). Phosphorous from the grain sample was determined as per Vanadomolybdo phosphoric yellow color method of Piper (1966) at the Department of Soil Science and Agricultural Chemistry, TNAU, Coimbatore. Finally, the iron and zinc estimation was done in Atomic absorption spectrophotometer following the method proposed by Jackson (1973) at the Department of Environmental Sciences, TNAU, Coimbatore. Utmost care has been taken to avoid soil and dust contamination in entire aforementioned process. The observations taken on seven grain nutritional quality traits (protein, crude fat, calcium, phosphorus, phytic acid, iron and zinc) and the mean values were subjected to statistical analysis. Analysis of variance was carried out by the method suggested by Panse and Sukhatme (1961). The significance test was carried out by referring to the 'F' table given by Snedecor (1961).

Results and Discussion

Per se performance of the parents is one of the simplest selection criteria for identifying superior genotypes. The genotypes with high *per se* performance would be much useful as parent for producing better offspring in any breeding programme (Saraswathi *et al.,* 1996; Singh and Singh, 1983). Pearl millet growing environment were characterized by short, erratic rainfall, high temperature and evapotranspiration and shallow sandy soil with poor water holding capacity. Although yield gains were sufficiently achieved, there are high genetic potentials for quality traits in pearl millet germplasm exist and untapped in breeding programmes. For instance, evaluation of pearl millet at ICRISAT showed large variation among the genotype for Fe (29-102 ppm) and Zn (24 -81 ppm) indicating good breeding prospects for its improvements. Hence, to investigate the range and magnitude of mineral content in available germplasm, an intensive research is needed across the globe. Keeping this in view, the present study has been focused to strengthen the micronutrient availability across the germplasm sources. The analyses of variance (data not presented) revealed highly significant differences between the genotypes for all the characters studied indicated that, presence of enough variability for all traits in germplasm.

In present study, the average protein content varied from 9.71 per cent (PT 5604) to 15.43 per cent (PT 5118). The grand mean accounted to12.24 per cent and twenty genotypes were found to excel the general mean significantly. (Table 1). Improvement of nutritional quality is presently being emphasized and considerable progress has been made towards genetic improvement of plant protein content. The need for genetic improvement of nutritional quality of pearl millet grain is emphasized by the fact that this crop is grown in arid regions whose populations face severe malnutrition (Mathur and Mathur, 1986). There is possibility to develop pearl millet cultivars, which are nutritionally superior with respect to protein content (Dodiya and Joshi, 2003). In the present investigation the promising genotypes for high protein content were PT 5118, PT 3718, PT 3657 followed by PT 2198, PT 2243, PT 2610, PT 4508, PT 4572, PT 5552, PT 5564, PT 5843, PT 5914 and PT 5188 (Y).

Pearl millet is a good source of lipids and useful for poultry feeds. Crude fat content in test germplasm varied from 2.55 per cent (PT 5099) to 6.95 per cent (PT 3758). Twenty nine genotypes showed significantly reduced crude fat content than the general mean. The flour quality of pearl millet depends upon the fat content. High fat content is attributed to poor storability and odour during the storage of flour makes it unacceptable for consumption. Thus the genotypes with low fat content are the desirable attributes in breeding for consumption. In this study PT 5099, PT 2659, PT 4377 were the promising genotypes having low fat content (2.5 to 3.0 per cent). Similarly, genotypes such as PT 3718, PT 4440, PT 5188 (Y), PT 5765 and PT 5864 were also found promising for this trait.

The range of phytate phosphorus varied from 135.29 mg (PT 1796) to 241.18 mg (PT 4219) and twenty six genotypes expressed significantly reduced level, than the grand mean. Diet, high in phytic acid and poor in calcium, iron and zinc produce deficiency symptoms in experimental animals (Gontzea and Sutzescu, 1968). Due to high content of phytic acid in pearl millet, the need to develop better cultivars with low phytic acid content can hardly be overemphasized. From the present study revealed that the genotypes *viz.,* PT 5554 and PT 5864 were found to be promising for low phytate phosphorus with high content of calcium, iron and zinc. The genotypes PT 1796, PT 5554 and PT 5552 had high iron and zinc content with low phytate phosphorus. PT 2198, PT 2582. The genotypes PT 4470, PT 4619 and PT 5188(Y) had high calcium content with low phytate phosphorus.

The phosphorus content of selected pearl millet genotypes ranged from 248.75 mg (PT 5547) to 372.63 mg (PT 4377). The grand mean recorded was 339.63 mg and seven genotypes exceeded the general mean significantly. Phosphorus is an essential component in nucleic acid and nucleo protein responsible for cell division, reproduction and transmission of hereditary traits. Perhaps, along with calcium this gives rigidity to bones and teeth of the body. The genotypes PT 2659, PT 3657, PT 4664, PT 5913, PT 5765 and PT 4760 had high phosphorus with considerably low phytate phosphorus. The calcium level in the grain varied from 24.66 mg (PT 5552) to 42.90 mg (PT 2835/1). Twenty three genotypes had significantly surpassed the grand mean of 35.91 mg. For iron content twenty four genotypes were found to excel the grand mean significantly, and for zinc twenty six genotypes were found to be exceeded significantly.

The ultimate aim of farmers to get remuneration from the farm and they will not prefer a cultivar with low yield potential, even though it is nutritionally superior. Breeder should always keep in mind that the yield potential should be in acceptable level and better than the existing genotype. Therefore, in present study highest grain yield of single plant

Table 1. Performance of sixty one pearl millet genotypes for grain nutritional quality characters

S. No.	Accession Name	Crude protein (%)	Crude fat (%)	Phytate phosphorus (mg)	Phosphorus (mg)	Calcium (mg)	Iron (mg)	Zinc (μg)	Grain yield per plant (g)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1	PT 1796	11.11	4.05	135.29*	279.8	28.5	5.02*	184.60*	61.57
2	PT 2198	13.30*	4.15	201.41*	355.85	40.60*	2.55	118.25	61.55
3	PT 2243	13.98*	3.25*	150.39*	296.75	32.55	3.75*	165.55*	59.38
4	PT 2582	11.99	3.15*	169.95*	306	42.85*	2.46	113.75	52.87
5	PT 2610	13.85*	3.05*	206.31	277.8	39.90*	2.31	120.6	59.04
6	PT 2659	12.96	2.63*	215.55	364.50*	32.8	3.53*	141.50*	57.57
7	PT 2835/1	12.55	4.4	236.25	356.2	42.90*	5.10*	195.93*	63.74
8	PT 3311	12.73	4.26	229.33	358.95	34.75	5.15*	188.05*	56.85
9	PT 3397	10.44	4.73	137.57*	260.5	35.1	3.49*	152.55*	59.85
10	PT 3488	9.99	3.85	240.33	362.7	28.75	3.53*	151.86*	52.17
11	PT 3559	11.1	4.36	209.77	355	41.95*	5.10*	181.60*	71.47*
12	PT 3657	14.80*	4.36	237.47	366.75*	30.58	2.3	115.63	59.35
13	PT 3687	11.02	3.85	213.26	346.55	30.56	3.61*	146.25*	60.03
14	PT 3718	15.20*	2.73*	219.38	360.6	32.7	2.68	151.45*	62.45
15	PT 3755	11.77	3.13*	224.35	362.7	35.77	2.93	116.02	51.52
16	PT 3758	10.39	6.95	197.36*	328.85	35.55	4.33*	151.60*	64.47*
17	PT 3764	9.92	4.43	214.38	341.87	35.66	5.46*	164.66*	58.59
18	PT 3987	13.60*	3.05*	218.36	359.28	34.25	2.68	142.01*	59.5
19	PT 4060	11.8	3.18*	211.47	334.66	34.61	2.59	115.8	53.83
20	PT 4219	13.48*	3.58*	241.18	361.71	29.95	2.96	136.65	60.77
21	PT 4266/2	11.25	5.53	239.52	362.77	32.8	2.46	112.83	62.12
22	PT 4377	9.88	2.70*	240.43	372.63*	35.73	2.75	122.15	58.09
23	PT 4440	13.39*	2.83*	235.77	362.5	40.50*	2.38	116.71	58.24
24	PT 4464	11.76	3.93	218.48	360.94	40.58*	1.88	112.51	61.69
25	PT 4470	11.22	4.05	203.48*	328.99	37.76*	2.36	114.76	59.63
26	PT 4508	14.56*	3.95	136.52*	316.75	33.66	5.60*	183.80*	62.65
27	PT 4551	12.14	4.05	216.68	342.83	34.48	3.38	151.75*	66.83*
28	PT 4572	14.19*	3.28*	227.45	360.05	38.57*	2.47	114.9	60.7
29	PT 4591	9.96	3.20*	200.63*	353.3	41.99*	3.81*	121.78	52.97
30	PT 4619	11.42	3.60*	205.76*	331.6	42.29*	2.69	113.72	53.22
31	PT 4664	13.26*	3.43*	240.07	364.85*	42.63*	5.63*	179.93*	60.45
32	PT 4760	12.85	6.83	210.93	366.65*	39.66*	3.05	114.63	73.15*
33	PT 4896	13.22*	6.9	229.53	361.76	41.65*	4.65*	131.66	77.22*
34	PT 4976	12.22	4.03	235	363.05	38.50*	3.19	141.50*	59.95
35	PT 5005	12.77	5.05	201.10*	320.6	36.75	2.37	117.81	64.18*

(79.17 g) was noticed in PT 5939 followed by PT 4896 (77.22 g) and the least grain yield per plant (51.52 g) was found in PT 3755 and thirteen genotypes were significantly higher than the grand mean. Considering the total variability contributed by different mineral content was not so impressive (Figure 1). However, among the minerals, phosphorus highly contributes (38%) towards total variability followed by zinc (26%) and phytate content (19%).

Table 1. Continued...

S. No.	Accession Name	Crude protein (%)	Crude fat	Phytate phosphorus (mg)	Phosphorus (mg)	Calcium (mg)	Iron	Zinc	Grain yield per plant (g)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
36	PT 5010/2	12.31	3.95	195.73*	344.6	36.5	1.7	112.76	63.42
37	PT 5021	9.78	4.08	236.04	361.55	39.55*	5.12*	186.95*	61.8
38	PT 5072	11.54	3.43*	198.29*	323.56	35.5	1.92	113.14	61.12
39	PT 5077	14.30*	4.68	215.33	354.6	36.5	2.28	127	68.54*
40	PT 5099	10.84	2.55*	213.11	353.6	40.55*	2.2	113.73	61.37
41	PT 5118	15.43*	3.36*	219.28	352.71	33.6	5.25*	156.78*	64.37*
42	PT 5179	10.23	6.63	204.30*	360.51	34.63	3.15	111.74	72.14*
43	PT 5188 (Y)	13.98*	2.80*	202.95*	321.05	37.50*	1.79	112.5	59.62
44	PT 5241	12.36	3.43*	201.65*	331.6	29.9	1.05	111.5	59.12
45	PT 5541	12.25	4.05	202.09*	324.8	30.5	1.73	121.62	59.83
46	PT 5547	12.21	3.36*	199.39*	248.75	27.61	4.62*	158.91*	62.67
47	PT 5552	14.45*	3.63*	201.02*	324.81	24.66	5.24*	184.55*	59.35
48	PT 5554	10.28	3.95	200.37*	351.63	41.12*	5.46*	169.01*	52.29
49	PT 5564	14.62*	3.05*	216.38	349.95	29.66	2.33	115.95	61.37
50	PT 5604	9.71	3.05*	204.37*	329.81	29.5	4.28*	159.50*	56.75
51	PT 5605	13.38*	3.84	235.39	363.83	31.5	2.01	123.77	62.54
52	PT 5625	10.57	3.37*	138.95*	269.92	31.56	2.07	131.5	52.25
53	PT 5722	11.28	4.05	212.3	353.34	36.5	3.06	130.63	63.57
54	PT 5744	10.03	6.02	215.83	363.29	39.59*	3.67*	141.62*	70.15*
55	PT 5765	11.58	2.99*	229.56	366.94*	40.63*	2.4	116.94	57.57
56	PT 5843	14.75*	3.10*	212.38	342.95	42.63*	3.44*	126.71	62.57
57	PT 5856	10.25	6.05	195.62*	316.8	35.61	2.78	116.78	76.77*
58	PT 5864	12.04	2.85*	147.77*	291.98	40.50*	4.28*	150.61*	58.95
59	PT 5913	12.65	3.78*	229.73	365.80*	36.62	2.6	118.6	60.45
60	PT 5914	14.73*	4.95	196.35*	321.7	34.63	2.11	113.79	70.59*
61	PT 5939	11.49	5.72	196.52*	321.27	35.81	3.29	151.25*	79.17*
	Minimum	9.71	2.55	135.29	248.75	24.66	1.05	111.5	51.52
	Maximum	15.43	6.95	241.18	372.63	42.9	5.63	195.93	79.17
	Mean	12.24	3.98	207.72	339.63	35.91	3.27	137.41	61.57
	S.E.	0.34	0.06	0.65	8.55	0.44	0.05	0.37	0.85
	C.D. at 5%	0.98	0.17	1.84	24.2	1.24	0.16	1.04	2.42

* Significance at 5% level; PT – *Pennisetum typhoides*

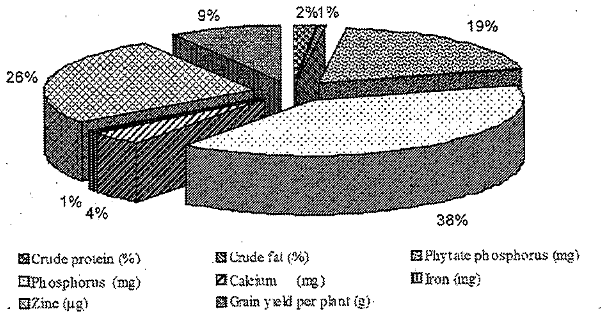

Figure 1. Percentage of total variability contributed by different mineral contents

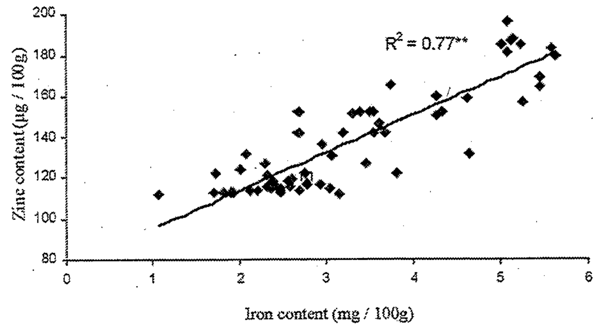

Figure 2. Relationship between grain iron and zinc content in pearl millet

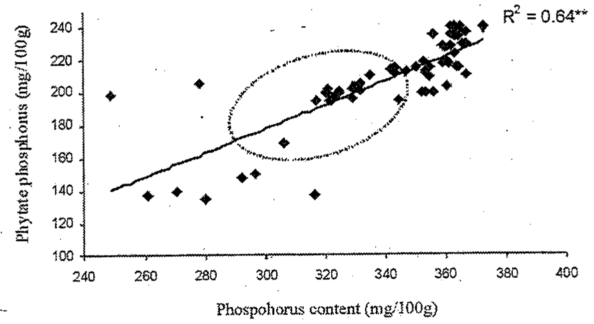

Figure 3. Relationship between grain total phosphorus and phytate phosphorus content in pearl millet

In present study, the relationship between grain iron and zinc content was significantly strong (r^2 = 0.77; P <0.001) and simple selection can enhance both the mineral levels simultaneously (Figure 2). The results are in line with the findings of Velu et al, (2007 and 2008). On the negative side, total phosphorus significantly associated with phytate (r^2 = 0.64; P <0.001), indicates the selection for low phytate will not operates since both are in positive directions but moderate to high phosphorus with considerable low level of phytate can be achieved through cautious selection (Figure 3). Present study was accordance with Sharma and Kapoor (2000). Biology of pearl millet has been studied little and much genetic resources still untapped (Rai *et al.* 1999), therefore attempts to improve biochemical and mineral content in upcoming varieties could significantly improve both the plants and the people who rely upon them. Further understanding the accumulation of these nutrients, functions and, the mechanisms will be essential to improve the quantity and quality of these minerals in plant foods.

Constraints in quality breeding: Breeding for mineral content is not so easy as any other trait. In general, there are some constraints such as traits are controlled by polygenes, which are highly influenced by environments, negative association of quality traits with agronomic traits in many crops and the quality character is mostly found in wild or related species. Transfer from wild species may pose cross incompatibility, hybrid inviability, hybrid sterility and linkage of undesirable genes with desirable ones. To overcome such limitations breeders and biologist evolved many advanced techniques such genomics and transformation. Still, a close interaction between the biochemist and breeders is lacking which sometimes becomes a limiting factor and progress becomes sluggish. The success of breeding for enhanced nutritional quality depends on three main issue *viz.*, (1) extent of genetic variability for the trait present in the gene pool (2) heritability of the character (3) analytical methods for quantitative estimation and close monitoring by breeders and nutritionist. Unless, these all factor not achieved concurrently we never ever produce a cultivar with elevated nutritional properties.

Breeding implications and future outlook: The micronutrient deficiency is widespread phenomena in dry land soils. Ability to grow well in dry and marginal soils with little or no irrigation and multiple uses for the grain rising pearl millet an attractive alternate crop. Recent efforts in cultivar development for grain production and nutritional attention have drawn to farmers. However, much work still remains ahead. Increasing public awareness about health benefit of pearl millet compared to other cereals and drawing industry's attention to its suitability for animal and bird feed will be essential for the making of large scale demand for the crop. The food based interventions is sustainable even external support fails and continuous in combating multiple micronutrient deficiency over the generations simultaneously. Pearl millet with large seed, tillering ability, and earliness coupled with elevated nutritional quality favour high demand. Improvement of crop through breeding procedures is dependent upon the presence of genetic variability and diversity among and within species. Selection for high yield and improved resistance for particular stress is well documented and for minerals yet to be done. It's believed that, germplasms are recognized as valuable genetic resources for improvement of any traits.

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