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Genetic variation for seed phosphorus and yield traits in Indian sorghum landraces and varieties

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ABSTRACT

Phytic acid is the major storage form of phosphorus in cereals. It binds with nutritionally important metals and affects mineral bioavailability. The present study analyzed phytic acid, inorganic phosphorus (IP) content, seed weight, and grain yield in 98 sorghum landraces and varieties grown in two environments to evaluate genotypic and environmental effects and to determine trait stability. Genotypic effects and genotype × interaction were significant for phytic acid concentration and yield components. A promising landrace, Malkhed-1, had the lowest phytic acid (0.015 mg g^{-1}) concentration, with a higher yield (70.02 g $plant^{-1}$), than the check variety M-35-1 in both environments. Similarly, among the varieties, Phule Maulee showed the lowest phytic acid (0.07 mg g^{-1}) and a higher grain yield of 53.15 g $plant^{-1}$ in both environments. Phytic acid and IP were negatively correlated ($r = -0.34$), whereas grain yield and seed weight were positively correlated ($r = 0.20$). Cluster analysis based on seed phosphorus traits and yield components identified five and six clusters, respectively. Genotypes containing low phytic acid with high yield identified in this study would be helpful for increasing the bioavailability of mineral nutrients.

1. Introduction

Sorghum is an important cereal grown mainly for food, feed, dietary fiber, and biofuel in subtropical and tropical Asia and Africa. In India it is cultivated on 7.89 Mha, of which 4.88 Mha is cultivated during the post-rainy season with a production of 4.18 Mt [1]. Cultivation of sorghum is concentrated mainly in peninsular and central India as a post-rainy season crop contributing 50% of total cereal intake. Sorghum is nutritionally superior to rice, as it supplies minerals, vitamins, protein, and micronutrients essential for health, growth, and development [2]. The presence of antinutritive factors, such as trypsin and amylase inhibitors and phytic acid, is known to interfere with protein, carbohydrate, and mineral metabolism. To improve the nutritional quality of sorghum and effectively exploit its potential as a food and feed crop, efforts should be made to reduce these antinutritive factors.

Phytic acid (myo-inositol hexaphosphoric acid, IP6) is the major phosphorus storage compound of most seeds and cereal grains, accounting for more than 70% of total

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phosphorus. Phytic acid (PAP) has a strong ability to chelate multivalent metal ions, especially zinc, calcium and iron. This binding can result in very insoluble salts with poor bioavailability of minerals [3]. Phytic acid is hydrolyzed enzymatically by phytases or chemically to lower inositol phosphates during storage, fermentation, germination, food processing, and digestion in the human gut [4]. The effects of phytic acid in human and animal nutrition are associated with the interaction of phytic acid with proteins, vitamins and minerals, thereby restricting their bioavailability [5]. Several methods have been employed to improve the nutritional quality of sorghum. Some of these methods, such as germination or sprouting, fermentation, soaking, dehulling, and cooking can drastically reduce the phytic acid content [6]. Low-phytic acid (*lpa*) mutants have been reported in soybean, maize, barley and rice [7].

Indian sorghum landraces possess moderate to high genetic variability, but their utilization in breeding programs for improving yield and seed quality has not been realized [8]. Assessment of genetic variability has accordingly become an essential component of identifying potential parents for recombination breeding. Quantitative traits such as phytic acid, inorganic phosphorus (IP), seed weight, and grain yield tend to differ from one environment to another. The interaction between genotype and environment has an important influence on the breeding behavior of the genotype. There is a need for extensive testing of these genotypes in varied agroclimatic conditions, for reducing the environmental influence. In this context, the present study aimed to estimate genetic variability for phytic acid, inorganic phosphorus, seed weight, and grain yield among sorghum landraces and popular varieties over two locations.

2. Materials and methods

The material used in this study comprised 83 sorghum landraces and 15 varieties including the popular check variety M-35-1, adapted to the post-rainy season in Karnataka, Maharashtra, and Andhra Pradesh states of India (Table S1). These genotypes were grown in two replications in a randomized complete block design at the Experimental and Gamma Field Facility, Bhabha Atomic Research Centre (E1), Trombay, Mumbai (19°03′ N; 72°93′ E) during the post-rainy season, 2013 and the Agricultural Research Station (E2), Gulbarga (17°36′ N; 76°81′ E), Karnataka state during the 2012 crop season. All agronomic practices were followed to produce an optimally healthy crop. Four quantitative characters: phytic acid, IP, grain yield per plant (g) and 1000-seed weight (g) were recorded for five randomly selected plants in both replications and locations. Plant yield was measured as the weight of the seed threshed from individual panicles. One thousand seeds were counted and weighed for each accession and recorded as the seed weight. For seed phosphorus estimation, selfed seeds from each genotype in each location were used.

2.1. Determination of phytic acid (PAP)

Phytic acid in sorghum was estimated by a modified colorimetric method [9]. A sample of 30–40 mg of ground seed was prepared from selfed seeds in each location in two replications. Ground samples (30 mg) were placed in an Eppendorf tube and 1 mL of 0.2 mol L^{-1} HCl extraction buffer was added and left overnight. Crude acid extracts were transferred to fresh tubes containing 20 mg NaCl. The contents were shaken at 3500 r min^{-1} for 20 min to dissolve the salt and allowed to settle at −20 °C for 20 min. The mixtures were centrifuged (8000 r min^{-1}) at 10 °C for 20 min and the clear supernatant was diluted 25 times by mixing with distilled water. Of this diluted sample, 750 μL was combined with 250 μL of modified Wade reagent (0.03% $FeCl_3{\cdot}6H_2O$ + 0.3% sulfosalicylic acid) in an Eppendorf tube, thoroughly mixed by vortexing, and centrifuged at 8000 r min^{-1} at 10 °C for 10 min. A series of calibration standards containing 0, 0.5, 1, 1.5, 2, 3, 4, 5, 7.5, 10, and 12 μg mL^{-1} of PAP were prepared from sodium phytate (Sigma, St. Louis, MO). The pink color of the Wade reagent is produced by the reaction between ferric ion and sulfosalicylic acid, with an absorbance maximum at 500 nm measured with a UV spectrophotometer (Thermo Electron Corporation). In the presence of phytate, iron is bound to the phosphate ester and is unavailable to react with sulfosalicylic acid, resulting in differential pink color intensity. The delta absorbance values were used to estimate phytic acid content and expressed in mg g^{-1} of the flour sample [10].

2.2. Determination of IP

Inorganic phosphorus (IP) was estimated colorimetrically using 30–50 mg of ground sample in two replications for each location separately. Ground samples were placed in an Eppendorf tube and incubated in extraction buffer [12.5% (v/v) trichloroacetic acid and 25 mmol L^{-1} $MgCl_2$] [11]. These samples were centrifuged at 10,000 r min^{-1} and the supernatant was diluted in a 1:2 ratio with distilled water. A 100-μL aliquot of the diluted sample was mixed with Chen's reagent [prepared by mixing 6 N H_2SO_4:2.5% ammonium molybdate:10% ascorbic acid:distilled water in a 1:1:1:2 (v/v/v/v) ratio] and incubated in a water bath at 50 °C for 1 h. After incubation, samples were cooled and absorbance was measured at 660 nm in a UV–vis spectrophotometer. A standard curve was plotted with the absorbance of known solutions of disodium hydrogen phosphate. Based on the calibration curve of the standard IP, the OD values of samples were converted to concentrations of IP and expressed in mg g^{-1} of sorghum flour.

2.3. Statistical analysis

Analysis of variance for PAP and IP concentrations, 1000-seed weight and grain yield per plant among the genotypes tested were computed with the SAS [12] procedure PROC GLM. Replication and locations were fitted as random effects and the fixed effects of genotypes were tested for significance. Summary statistics, genotypic and phenotypic coefficients of variation [13], heritabilities [14] and correlation coefficients were calculated for each of the traits. Cluster analysis was performed to evaluate associations among the genotypes based on the seed "P" and yield traits. All the above statistical analyses were performed using SAS.

Table 1 – Analysis of variance for seed phosphorus, seed weight, and grain yield in sorghum genotypes.

Source	*df*	Mean squares			
		Phytic acid	IP	Yield/plant	1000-seed weight
Genotype	97	4.170**	0.338**	0.491**	270.94**
Environment	1	0.0001	0.082*	14.043**	5989.41**
Rep (env.)	2	0.007	0.001	0.231	8.93
G × E	97	0.042**	0.012	0.406**	240.49**
Error	194	0.004	0.009	0.037	2.95
CV (%)		6.12	23.42	5.03	5.98
CD at 5%		0.20	0.29	5.30	0.61

Significance at $*P < 0.05$; $**P < 0.01$. CV: coefficient of variation; CD: critical difference.

Table 3 – Correlation coefficients for seed phosphorus and yield traits in sorghum genotypes.

Trait	Phytic acid	IP	1000-seed weight
Phytic acid	1		
IP	−0.345**	1	
SW	−0.077	0.102*	1
Yield/plant	−0.088	0.093	0.202**

Significance at $*P < 0.05$ and $**P < 0.01$.

3. Results

3.1. *Evaluation of yield and seed phosphorus traits*

The analysis of variance showed a significant variation among the genotypes for phytic acid, IP, grain yield, and 1000-seed weight (Table 1). G × E interaction was highly significant for PAP, grain yield, and 1000-seed weight. The frequency distribution of phytic acid content among the genotypes showed that most (65) of the genotypes possessed low PAP values, ranging from 0.02 to 1.00 mg g^{-1}. Only 35 genotypes had high PAP values (3.2–4.3 mg g^{-1}). In the E-1 environment, a wide range of values were observed for grain yield (4.25–77.25 g plant^{-1}) with a mean value of 30.15 g plant^{-1}; 1000-seed weight (21.05–35.03 g) with mean of 30.49 g (Table 2). For seed phosphorus traits, PAP showed a wide range of values (0.015–4.400 mg g^{-1}) with a mean of 1.08 mg g^{-1} and IP ranged from 0.007 to 1.327 mg g^{-1} with a mean of 0.39 mg g^{-1}. Similarly in the E-2 environment, a wide range of values was observed for grain yield (24.6–59.9 g), 1000-seed weight (23.54–35.08 g), phytic acid (0.04–4.20 mg g^{-1}), and IP (0.013–1.500 mg g^{-1}). In both environments, the landrace Malkhed-1 showed the lowest phytic acid (0.015 mg g^{-1}) with high IP (0.70 mg g^{-1}) followed by Nalwar-2. Among the popular varieties, Phule Maulee showed the lowest phytic acid (0.07 mg g^{-1}) and highest IP (1.35 mg g^{-1}). The landrace Tengalli-2 showed the highest grain yield of 77.25 g plant^{-1} with a 1000-seed weight of 35.30 g, representing a 167% increase over the check variety M-35-1 in the E-1 environment. In the E-2 environment, the PC-6 variety showed the highest grain yield of 59.90 g plant^{-1}, and the Mangalagi-1 landrace was a moderate yielder (35.08 g) but had bold seeds.

3.2. *Estimation of genetic parameters*

Phytic acid showed the highest GCV (genotypic coefficient of variation) (93.97% and 88.98%) and seed weight the lowest GCVs (11.36% and 17.15%) compared with the other traits, in locations E1 and E2, respectively (Table 2). Location E1 showed lower values than location E2 for GCV and PCV (phenotypic coefficient of variation) for all traits except 1000-seed weight, whereas the GCV and PCV values for 1000-seed weight were highly variable, representing a location effect. High heritabilities (97.45% and 95.38%) were observed for grain yield in both locations, whereas 1000-seed weight had lower heritability (64.89) in location E1 (Table 2). Correlation estimates for the sorghum genotypes over the two environments indicated that PAP and IP were negatively correlated ($r = -0.345^{**}$) (Table 3, Fig. 1). Grain yield was significantly correlated with seed weight ($r = 0.202^{**}$). Grain yield and seed weight were negatively but non-significantly correlated with PAP (−0.088 and −0.077, respectively) but positively and non-significantly correlated with IP (0.093 and 0.102).

Table 2 – Mean, range and genetic variability components for seed phosphorus and yield traits in sorghum genotypes.

	Range	Mean	V_g	V_p	GCV	PCV	h^2
E1							
Phytic acid	0.015–4.400	1.08	1.03	1.14	93.97	98.64	90.75
IP	0.007–1.327	0.39	0.08	0.09	73.12	77.57	88.87
Yield/plant (g)	4.25–77.25	30.15	192.09	197.12	45.97	46.57	97.45
1000-seed weight (g)	21.05–35.30	30.49	11.99	17.66	11.36	13.78	67.89
E2							
Phytic acid	0.04–4.20	1.08	0.92	0.97	88.98	91.41	94.75
IP	0.013–1.500	0.42	0.09	0.10	72.09	74.76	93.00
Yield/plant (g)	24.60–59.91	37.98	55.13	57.80	19.55	20.02	95.38
1000-seed weight (g)	23.54–35.08	31.49	29.17	30.89	17.15	17.65	94.43

V_g and V_p: genetic and phenotypic variances; GCV and PCV: genetic and phenotypic coefficients of variation; E1: BARC, Mumbai, E2: ARS, Gulbarga. h^2: broad-sense heritability.

3.3. Cluster analysis

Cluster analysis was performed for seed phosphorus and yield components of 98 genotypes using paired-group and Euclidean similarity measures. Cluster analysis for the seed phosphorus traits resolved all the landraces and varieties into five clusters (Fig. 2). Most of the landraces showing low phytic acid, including Malkhed-1 (TSG-52), Mangalagi-1 (TSG-53), Mangalagi-3 (TSG-55), and Nalwar-2 (TSG-62), were grouped in cluster III. The mean phytic acid values for these genotypes were lowest, ranging from 0.015 to 0.400 mg g^{-1}. Similarly, six clusters were formed for 1000-seed weight and grain yield traits (Fig. 3). The PC-6 (TSG-93) variety and the Tengalli-2 (TSG-80) landrace diverged from the rest of the genotypes and formed a separate cluster with yield levels ranging from 68.0 to 77.25 g $plant^{-1}$. Cluster II grouped most of the high-yielding and bold-seeded genotypes (with a range of 41.52–70.02 g $plant^{-1}$ and 23–34 g, respectively). Most of the moderately high-yielding and medium-sized seeds were grouped in clusters III, IV, V, and VI.

4. Discussion

The relative performances of cultivars for quantitative traits such as seed weight, grain yield, and seed phosphorus vary from one environment to another. To develop a variety with high yielding ability and consistency, focus should be placed on the multi-environment testing of genotypes and precise estimation of their interactions. The interaction between genotype and environment has an important bearing on the stability of varieties [15]. The magnitude of genetic expression and of trait associations is important for the prediction of response to selection in diverse environments and provides the basis for planning breeding programs. The results of the present study in two locations (Mumbai and Gulbarga) indicated the presence of significant variability for seed phosphorus and yield traits. A wider range of values was observed for PAP, IP, seed weight, and grain yield in the landraces than in the varieties over both the locations. The traditional landraces were not bred for seed phosphorus, but for grain yield. Owing to their wide adaptability under varied agroclimatic conditions and different soil fertility levels, one could expect wide variability for seed phosphorus in landraces. The E-1 location is in the central Indian agroclimatic zone, characterized by shallow soils with a heavy rainfall. E-2 location is in southern India, characterized by deep black soils (Vertisols) with high water holding capacity and low to moderate rainfall. With this marked difference in agroclimatic conditions, the phenotypic expression levels are highly variable. As shown in the present study, there was significant G × E interaction for phytate concentration, grain yield and 1000-seed weight ($P < 0.001$). Raboy and Dickinson also found significant environmental effects for PAP, which they concluded were due to soil phosphorus availability in soybean [16]. Hence, soil characteristics should be considered in breeding for low phytic acid. In our studies, environmental effects were significant only for PAP and not for IP (Table 1). Some of the promising landraces, namely Tengalli-2, Nalwar-2, and Kannur-4, were exceptionally superior for the seed phosphorus traits and per se performance. Similarly, popular varieties such as M-35-1 (control), PC-6, and Phule Maulee equally outperformed landraces in both the environments.

Methods employed to improve the nutritional quality and organoleptic properties of cereal-based foods include genetic improvement, amino acid fortification, supplementation or complementation with protein rich sources, and processing technologies that include milling, malting, fermentation, or sprouting [17]. Attempts to reduce phytate content have employed different means including milling [18] and soaking and fermentation of sorghum grains [19,20] and activation of indigenous phytase and/or addition of microbial phytase [21]. In the present study, mean phytic acid was drastically reduced from 0.523 mg g^{-1} (control) to 0.027 mg g^{-1} (TSG-30) and IP from 0.331 mg g^{-1} (control) to 0.009 mg g^{-1} (TSG-13).

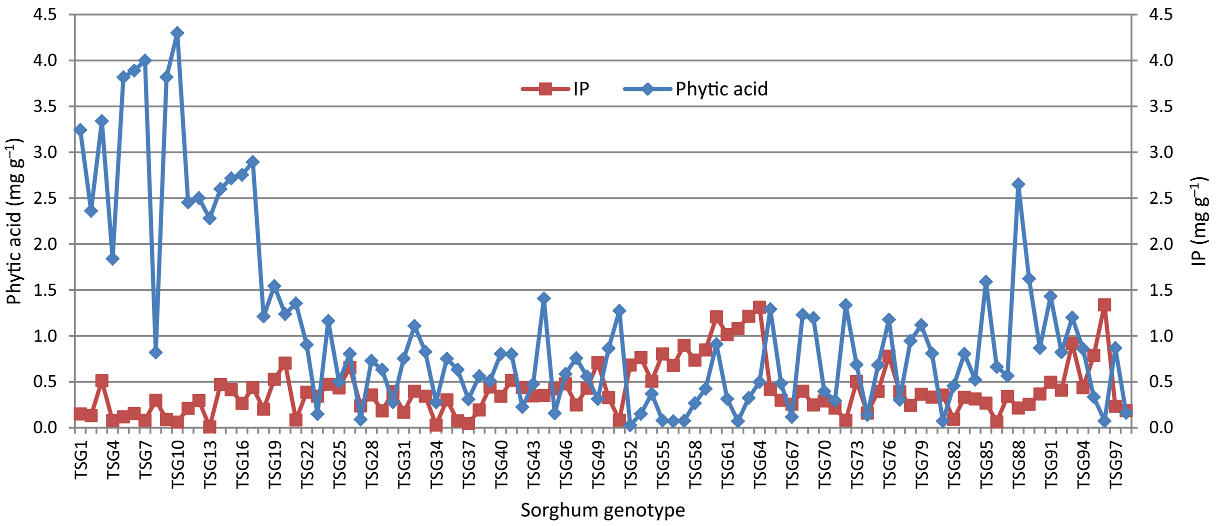

Fig. 1 – Genetic variability among sorghum landraces and varieties for phytic acid and IP over two locations [X axis, sorghum genotypes; Y1 axis, phytic acid (mg g^{-1}); Y2 axis, IP (mg g^{-1})].

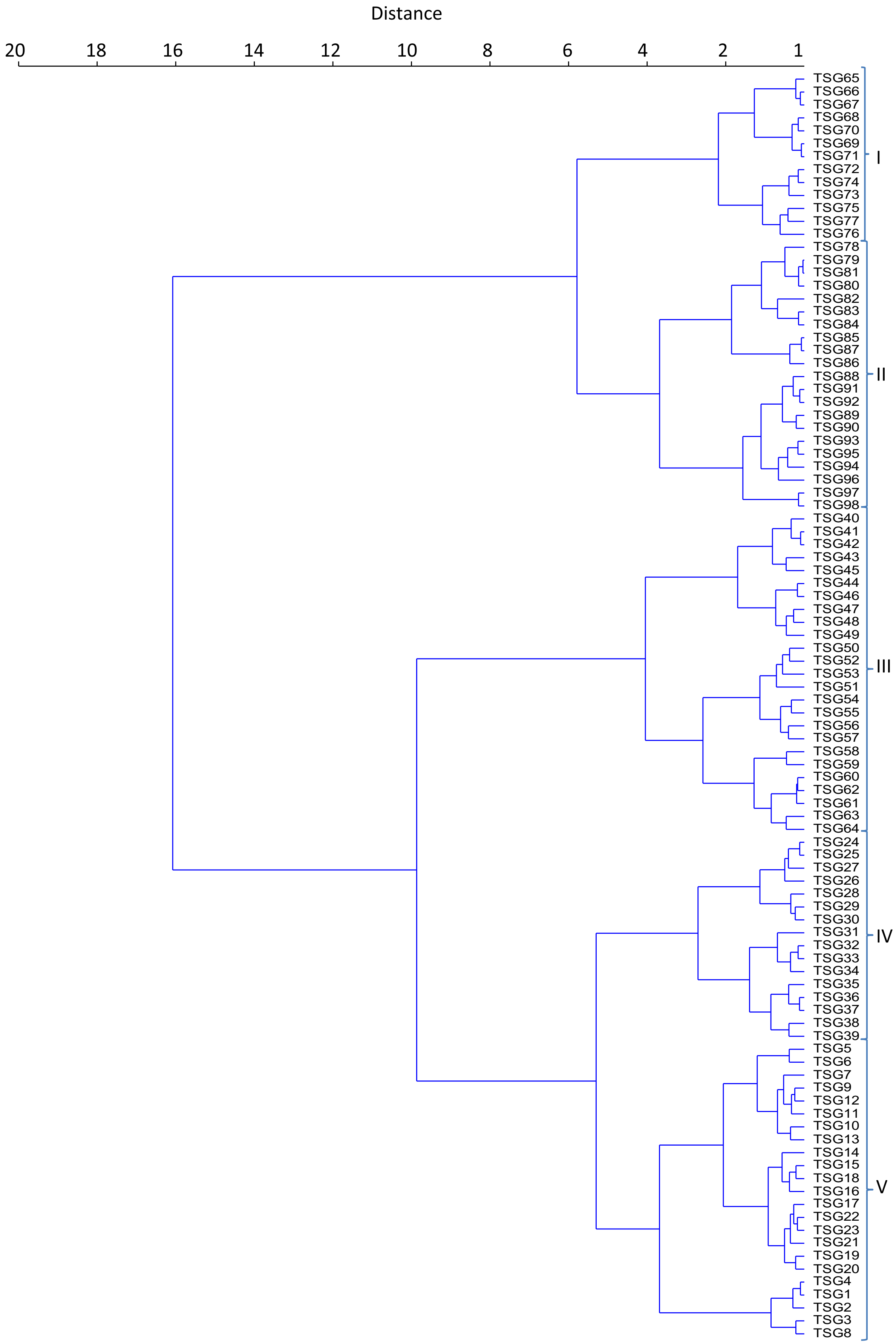

Fig. 2 – Cluster analysis for phytic acid and IP in sorghum landraces and varieties grown across two locations.

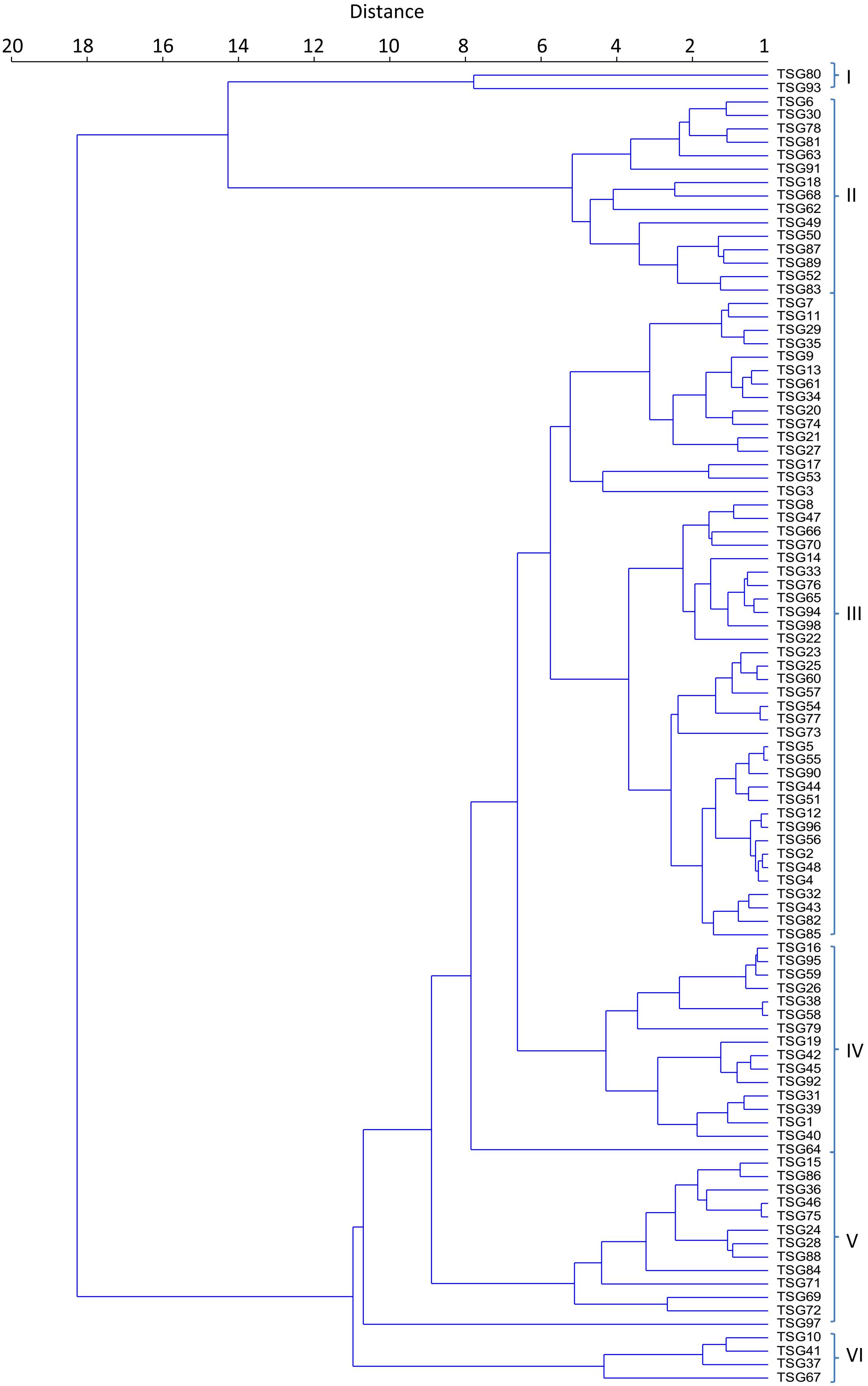

Fig. 3 – Cluster analyses for 1000-grain weight and grain yield in sorghum landraces and varieties grown across two locations.

Despite this drastic reduction, germination and seedling growth were not affected. Earlier report indicated phytate levels in traditional and improved whole sorghum flours to be 4.03 and 7.26 mg g^{-1}, respectively [22], which were much higher than the values recorded in the present study. A systemic reduction of phytic acid levels has negative effects on germination, emergence, and seed filling [23]. But field-grown soybean with low (0.09 mg $seed^{-1}$), medium (0.59 mg $seed^{-1}$) and high (1.00 mg $seed^{-1}$) phytic acid showed normal seedling development. This finding indicated that seedlings normally contain a phosphorus reserve far above that needed for germination and early growth of the plant [23]. Even silencing the expression of an ATP binding cassette (ABC) transporter in an embryo-specific manner generated low phytic acid and high-inorganic-phosphorus transgenic maize seeds [24] that showed normal germination and no significant reduction in seed dry weight.

The PCV was higher than the GCV for all the traits studied, suggesting that the environment had a little effect on the expression of these traits. The GCV provides a measure for comparing genetic variability in quantitative traits. The seed quality traits such as, phytic acid and IP, showed the highest GCV and PCV values. But GCV together with heritability estimates gives a good inference of the extent of heritable variation [25]. Accordingly, phytic acid and IP showed high GCV and heritability values compared with the yield-contributing traits. The high estimates of heritability for PAP (90.75 and 94.75) and grain yield (97.45 and 95.38) in the E1 and E2 locations have been found useful in plant breeding, as they enable the selection to be based on phenotypic performance [26].

Correlations between traits are of great importance for the success of selections to be conducted in breeding programs. In the present study, seed weight and grain yield were significantly correlated, indicating that simultaneous selection for these yield components is possible. Knowledge of the existing phenotypic and genetic variation and their association with heritability is of interest, because it allows simultaneous selection of two or more traits. Sorghum landraces have not been subjected to any systematic selection or breeding apart from traditional farming practices. Thus, the efficiency of improvement of such landraces may be enhanced by the identification of morpho-physiological traits associated with better yield response [27]. Phytic acid phosphorus and IP were significantly negatively correlated in the present study, suggesting the improvement of either one of these traits in a genotype. Varietal effects appeared to be the most critical factor in selecting a sorghum variety for human consumption that would contain optimum levels of available phosphorus. The strong negative correlation between PAP and IP makes it unlikely that non-phytic phosphorus would be increased by drastic reduction of phytic acid [28]. Thus, there is a need to balance the two forms to avoid interfering with germination. Cluster analysis based on location means for seed phosphorus and yield traits showed close relationships between landraces and popular varieties showing low phytic acid in a high-yielding background. Malkhed-1, Mangalagi-1, Mangalagi-3, and Nalwar-2 were promising landraces in cluster III with lower phytic acid content than the control. Phule Maulee, a shootfly-tolerant variety, showed lower phytic acid than M-35-1. For the yield components, six clusters were identified, with PC-6 and Tengalli-2, showing a high grain yield, forming a separate cluster.

Use of sorghum as food for human nutrition is constrained by a high level of antinutritive factors, in particular phytic acid, which can reduce the bioavailability of trace elements. Genetic improvement as well as pre-treatment methods such as fermentation, soaking, or germination improves nutritional quality, particularly through the breakdown of antinutritive factors. Using genetic and mutation breeding principles, *lpa* mutants have been identified in several crops with the aim of improving phosphorus and mineral bioavailability [29]. Low-phytate crops show enhanced bioavailability of phosphorus and several important nutritional cations including iron. Low-phytic acid landraces identified in the present study proved to be consistent across two different agroclimatic regions for seed phosphorus and per se performance. They can be used in recombination breeding to develop tailor-made varieties/hybrids showing improved mineral bioavailability in a high-yielding background.

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Supplementary material

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