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Research Article

Genetic variability for phytic acid and inorganic phosphorous in Indian Sorghum (*Sorghum bicolor*) landraces

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Abstract
Majority of the phosphorous in the seeds of higher plant is stored as phytic acid. Phytate 'P' interfere in the protein digestion and chelate nutritionally essential elements, such as Ca, Zn and Fe. Breeding for low phytic acid would help in improving the nutritional quality of sorghum. In the present study, genetic variability for phytic acid (PAP) and inorganic 'P'(IP) was determined among 92 sorghum landraces and 20 varieties.. The ANOVA showed significant differences for grain yield, 100-seed weight, PAP and IP among these genotypes. Wide range values were observed for grain yield (2.5-76.5 g/plant), 100-seed weight (2.06-4.1 g), PAP (0.015-4.450 mg/g) and IP (0.006-1.320 mg/g). Land race Malkhed-1 recorded high yield (69.03 g) with the lowest PAP values for phytic acid (0.015mg/g) and 0.67 mg/g of IP with IP/PAP ratio of 43.94. Correlation studies indicated that PAP and IP were negatively correlated (r = - 0.34). Cluster analysis based on the grain yield and seed 'P' traits grouped 112 genotypes into five clusters. Landrace Tengalli-6 was found highly diverse compared to rest of the genotypes. High yielding genotypes with low phytic acid identified in this study would be helpful in increased bioavailability of mineral nutrients.

Key words:
Sorghum, phytic acid, genetic variability, correlation

Introduction:

Sorghum is one of the important cereal crop grown for food, feed and industrial purposes. India ranks first in area (7.67 mha) and second in grain production (6.98 mill. tonnes) worldwide (Kannababu *et al.,* 2013). It is widely grown in rainfed areas of Central and South Indian states which contribute 50% of the total cereals intake. Sorghum is nutritionally superior to rice, as it supplies minerals, vitamins, protein and micronutrients essential for health, growth and development (Chan *et al.,* 2007). Some of the nutritional components (protein and minerals) are less bio-available due to anti nutritional factors such as phytic acid. They interfere in the protein digestion or chelate nutritionally essential elements including Ca, Zn and Fe (Hurrel *et al.,* 2003). In order to improve the nutritional quality of sorghum and effectively utilize its potential as food and feed crop, efforts should be made to reduce phytate content.

Phytic acid is widely distributed in nature because it is a major storage of phosphorous (P) in cereals, legumes and oil seeds (Harland and Oberleas, 1987). It is typically found in outer aleurone layer of cereal grains and in the endosperm of legumes and oilseeds. Majority of the 'P' (70%) in the seeds of higher plant is stored as myoinositol 1,2,3,4, and 6 hexakisphosphate or phytic acid. It is mainly present as a salt of mono-valent and divalent cations such as K^+, Mg^{2+} and Ca^{2+}. Phytate is naturally formed during maturation of plant seeds and grains and thus forming a common constituent of plant derived food. Based on the food intake and level of processing, daily intake can be as high as 4500 mg (Reddy, 2002). Phytate behaves as a negatively charged ion in a broad pH range and has a high affinity for minerals, trace elements and proteins. But phytate-phosphorus (PAP) is less nutritionally available since the phytate is not quantitatively hydrolysable in human gut (Sandberg and Anserson, 1988). High phytic acid content in animal feeds is generally supplemented with inorganic phosphate, as it causes increased fecal phosphate and subsequent eutrophication of water bodies. In addition, ameliorating with commercial phytases is also becoming popular and reduces the requirement for inorganic phosphate supplementation, releasing inorganic 'P' and myoinositol. Phytic acid in the whole grain is maximum and could be reduced by dehulling, grinding, soaking and cooking (Reddy *et al.,* 1982). It could completely be degraded in weaning cereals by adding commercial exogenous phytases or by activating the native phytases by soaking, germinating and fermentation (Marero *et al.,* 1991). Recently, mutagenesis and transgenic approaches have been used to generate low phytic acid genotypes, which are unavailable in germplasm resources (Wilcox et al., 2000). Low phytic acid (lpa) mutants were reported in soybean, maize, barley and rice. They block the ability of a seed to

synthesize 'P' in the phytic acid (Rayboy *et al.*, 2001; Shi *et al.*, 2012).

Local sorghum landraces with desirable characteristics such as wider adaptability, good grain quality, are highly preferred by consumers and thus play a significant role in local economies (Reddy *et al.*, 2012; Nkongolo and Nsapato 2003). Indian sorghum landraces possess moderate to high genetic variability but their subsequent utilization in the breeding program for improving the yield and seed quality has not been reached to an appreciable level (Reddy *et al.*, 2012). Assessment of genetic variability therefore becomes an essential component in identifying potential parents for recombination breeding. As per the literature available, the phytic acid composition of sorghum landraces and Indian cultivars has not been studied to meet the nutritional requirements for human/animal consumption. The objectives of this study were to estimate genetic variability for phytic acid and inorganic 'P' among popular sorghum varieties and historical landraces.

Material and Methods

The material used in this study comprised of 92 sorghum landraces and 20 varieties including popular check variety M-35-1 adapted to post rainy season from Karnataka, Maharashtra and Andhra Pradesh states of India (Table 1). These genotypes were grown in two replications in Randomized Complete Block Design (RCBD) at Experimental and Gamma Field Facility, Bhabha Atomic Research Centre, Mumbai during post rainy season, 2012. All the agronomic practices were followed to raise the ideal and healthy crop. Selfed seeds were harvested from each genotype and replicated grain samples (20 g) were used for biochemical assays.

Determination of phytic acid (PAP): Phytic acid estimation in sorghum was performed by following modified colorimetric method (Vaintraub, and Lapteva, 1988). About 30-40 mg of ground seed sample was used for extraction of phytic acid in 0.2 N HCl buffer and kept overnight. Crude acid extracts were transferred to fresh tubes containing 20 mg NaCl. The contents were shaken at 350 rpm for 20 min. to dissolve the salt and were allowed to settle at –20°C for 20 min. The mixtures were centrifuged (8000 rpm) at 10°C for 20 min. and clear supernatant was diluted 25 times by mixing with distilled dH_2O. 750 µl of this diluted sample were combined with 250 µl of modified Wade reagent (0.03% $FeCl_3.6H_2O$ + 0.3% sulfosalicylic acid) in an eppendorf tube, thoroughly mixed by vortexing and centrifuged at 8000 rpm 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 of PAP were prepared from sodium phytate (Sigma, St. Louis, MO). The pink color of the Wade reagent is due to the reaction between ferric ion and sulfosalicylic acid with an absorbance maximum at 500 nm using UV spectrophotometer (Thermo Electron Corporation). In the presence of phytate, the iron was bound to the phosphate ester and was 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 of the flour sample (Latta and Eskin, 1980).

Determination of Inorganic Phosphorous (IP): Inorganic P was estimated colorimetrically using 30-50 mg of a ground sample in 12.5% (v/v) dissolved in Tri Chloro Acetic acid and 25mM $MgCl_2$ buffer (Chen *et al*., 1956). Overnight incubated samples were centrifuged at 10,000 rpm and supernatant was diluted in 1:2 ratios with distilled water. A 100 µl of the diluted sample was mixed with Chen's reagent and incubated in water bath at 50°C for 1h. After incubation, samples were cooled and absorbance was taken at 660 nm in a UV-Vis spectrophotometer (Thermo Electron Corporation). A standard curve was plotted by taking the absorbance of known amount of disodium hydrogen phosphate. Based on the calibration curve of the standard inorganic P, the respective OD value of a sample was converted to concentration of inorganic P and expressed in mg/g of the sorghum flour.

Statistical analysis: Analysis of variance for PAP and IP concentration, 100 seed weight and grain yield per plant among the genotypes tested was computed using SAS procedure, Proc GLM (SAS, 2010). Replication was considered as random effect and fixed effect of genotypes were tested for significance. Summary statistics (univariate) and genetic variability components were calculated using PAST software (Hammer et al., 2001). The PROC GLM procedure was used to estimate variance for all the traits. Genotypic and phenotypic coefficient of variation (Burton, 1952), heritability (Allard 1960) and genetic advance (Johnson *et al.*, 1955) were calculated for each of the traits. The SAS procedure PROC CORR (SAS, 2010) was used to calculate the correlation coefficient between biochemical and yield components. The Euclidean distances were used to construct dendrogram in PAST software.

Results and Discussion

In the present study, 112 genotypes were used to estimate yield, 100 seed weight, PAP and IP (Table 1). The analysis of variance showed significant differences for these traits (Table 2). Wide range values were observed for grain yield (2.5-76.5 g/plant) with mean value of 31.1 g/plant (Table 3). The landrace, Tengalli-6 recorded the highest grain yield of 76.5 g/plant, which was 165% increase over check variety M 35-1. Other high yielding

landraces were Malkhed-1 and Bommanalli. Similarly, 100 seed weight (2.06-4.1 g) was also registered wide range values across the genotypes. None of the landraces had larger seed than the check variety, M-35-1 (4.01g). However, some varieties like Phule Revathi, and landraces, Tengalli-6 and Kannur 1-1 (4 g) showed comparatively higher seed weight.

Both PAP (0.015 – 4.45 mg/g) and IP (0.006 – 1.32 mg/g) showed wide range values among the sorghum genotypes. Landrace, Chicknagur recorded the highest PAP (4.45 mg/g) with 0.06 mg/g of IP values and IP/PAP ratio of 0.014. While Malkhed-1 recorded the lowest PAP of 0.015 mg/g with 0.67 mg/g of IP and IP/PAP ratio of 43.94. Among the varieties, Phule Maulee recorded 0.078 mg/g of PAP and 1.321 mg/g of IP with IP/PAP ratio of 17.0. The accumulation of PAP would depend on factors that affect uptake of P such as differential status of soils, soil pH, temperature and P mineralizing micro organisms in the soils (Israel et al., 2007). Wide range values were observed for PAP (0.02-4.45 mg/g) and grain yield (2.5-76.5 g) in the landraces as against varieties. The traditional landraces were not bred for seed phosphorous, instead for grain yield. Due to their wide adaptability under varied agro-climatic conditions and different soil fertility levels, one could expect wide variability for seed P in landraces. PAP recorded the highest GCV (98.38%) compared to rest of the traits. Overall, there were narrow differences observed between PCV and GCV for most of the traits under study. The extent of variability should be transferred to the next generation, so as to effectively implement selection. Heritability is one such criterion to gauge the effectiveness of breeding for quantitative traits. High heritability values were observed for IP (99.52%); grain yield (98.48%) and PAP (97.43%). In breeding for quantitative traits, high heritability with high genetic advance could be due to more additive component, thus facilitating selection based on *per se* performance.

Correlation studies indicated that PAP and IP were negatively correlated (r = - 0.34**) indicating low PAP genotypes were with high IP and vice versa (Fig. 1). Rayboy *et al.,* (2000) reported gradual PAP accumulation during seed development and decrease in IP concentrations as grain matures. It has been shown that phytic acid levels were correlated with the supply of P to the plant and with the content of inorganic phosphorus in leaves (Raboy and Dickinson, 1993), which ultimately lead to increased translocation of 'P' to the grain. Cluster analysis based on the seed yield and 'P' traits grouped the entire 92 landraces and 20 popular varieties into five clusters (Fig. 2). TSG 77 (Tengalli-6) landrace found highly diverse compared to rest of the genotypes. This genotype was high yielding (76.5 g/plant) with larger seeds (4.0 g) and moderate 'P' values (0.82 mg/g of PAP). Cluster II comprised of 27 landraces and 8 varieties, which showed moderate levels of seed 'P' and yield levels. Most of the genotypes were genetically narrow for the seed P and yield traits. Popular varieties were scattered in all the clusters, as they had been developed using local landraces.

The methods employed to improve the nutritional quality of cereal-based foods include genetic or biotechnological approaches and several pre-treatment methods such as fermentation, soaking, germination (Chavan and Kadam, 1989). Germination is a process widely used in legumes and cereals to increase their palatability and nutritional value, particularly through the breakdown of certain anti-nutrients, such as phytate. Reducing the phytate content have been tried by different means including milling (Mahgoub and Elhag, 1998) and soaking of sorghum grains (Elmaki *et al.,* 1999), fermentation of sorghum, maize and soybean (Marfo *et al*., 1990) and activation of the indigenous enzyme phytase and/or addition of microbial phytase (Barrier et al., 1996). Using genetic and mutation breeding principles, lpa mutants have been identified in several crops in order to improve the 'P' and mineral bioavailability (Rayboy *et al*., 2001.). Low phytate crops have several benefits, as they enhance the bioavailability of 'P' and several important nutritional cations including iron (Warkentin *et al*., 2012). The present study identified few low phytic acid landraces with good yielding ability. They can be used in recombination breeding, so as to develop tailor made varieties/hybrids, such that mineral bio availability could be improved.

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Table 1. Mean values for the seed weight, phytic acid, IP and grain yield among the sorghum landraces/ varieties selected for the study

Code	Genotypes	100 Seed weight (g)	Phytic acid (mg/g)	IP (mg/g)	Yield/pl (g)
Local landraces					
TSG1	Afzalpur-1	3.60	3.674	0.128	38.50
TSG2	Afzalpur-3	3.40	2.139	0.119	15.00
TSG3	Athannur-1	3.16	3.424	0.425	20.00
TSG4	Aurad	3.30	1.943	0.076	14.50
TSG5	Balaganur-2	2.84	4.272	0.112	23.15
TSG6	Bommanalli	2.88	4.004	0.112	62.35
TSG7	Athanur-3	2.46	4.147	0.074	18.30
TSG8	Chavadapur-2	2.64	4.058	0.094	15.50
TSG9	Chicknagur	2.90	4.451	0.060	21.50
TSG10	Chincholli-1	2.84	2.344	0.144	21.80
TSG11	Chincholli-2	3.27	2.559	0.225	18.70
TSG12	Chincholli-1-2	3.08	2.202	0.006	21.80
TSG13	Chittapur Maldandi-3	3.76	2.362	0.538	18.50
TSG14	Chittapur Maldandi -4	3.40	2.996	0.428	18.00
TSG15	Chittapur Maldandi -5	2.92	2.817	0.232	24.30
TSG16	Chittapur Maldandi -9	3.82	2.880	0.480	20.00
TSG17	Chittapur-1	2.60	1.158	0.175	61.70
TSG18	Dhamapur	2.92	1.595	0.459	23.95
TSG19	Gavar-1	3.30	1.193	0.694	21.80
TSG20	Gola-1	3.56	1.309	0.083	14.07
TSG21	Gola-3	3.26	0.997	0.376	23.50
TSG22	Gola-4	3.44	0.149	0.315	21.40
TSG23	Gola-5	2.90	1.006	0.448	32.13
TSG24	Gotur-1	3.40	0.444	0.446	21.50
TSG25	Gulbarga-2	2.94	0.095	0.177	20.70
TSG26	Gundurgthi	2.86	0.515	0.171	10.33
TSG27	Hagargi-2	2.06	0.738	0.144	21.10
TSG28	Hagatti-2	3.28	1.015	0.344	22.60
TSG29	Hattigudur	3.02	0.854	0.324	29.50
TSG30	Hattigudur-1	3.00	0.274	0.020	29.05
TSG31	Hattigudur-6	2.52	0.738	0.205	19.78
TSG32	Hebbal-1	3.35	0.774	0.080	25.10
TSG33	Hebbal-2	2.86	0.319	0.051	18.20
TSG34	Hebbal-5	2.40	0.506	0.092	26.70
TSG35	Hebbal-6	3.06	0.506	0.389	40.50
TSG36	Ingalagi-2	2.34	0.854	0.326	31.17
TSG37	IS-2293*	2.14	0.801	0.579	18.98
TSG38	IS27036*	2.70	0.212	0.500	39.00
TSG39	Sirgapur	2.80	0.390	0.225	25.20
TSG40	Sonnur	2.56	1.274	0.083	25.10
TSG41	IS-6920*	2.92	0.479	0.324	21.60
TSG42	IS-7530**	3.10	1.515	0.302	24.35
TSG43	Kalkora	3.10	0.140	0.495	36.00
TSG44	Kannur-1	3.40	0.604	0.453	42.20
TSG45	Kannur-1-1	4.00	0.095	0.619	38.20
TSG46	Kannur-2	3.28	0.720	0.198	25.60
TSG47	Kannur-3	3.07	0.515	0.455	21.20
TSG48	Kannur-4	3.76	0.229	0.728	41.03
TSG49	Kodekal-2	3.06	1.256	0.083	21.50
TSG50	Malkhed-1	2.80	0.015	0.667	69.03
TSG51	Mangalagi-1	3.56	0.104	0.725	12.30
TSG52	Mangalagi-2	3.88	0.354	0.516	21.50
TSG53	Mangalagi-3	3.20	0.078	0.908	24.40
TSG54	Mangalagi-4	3.52	0.069	0.653	28.78
TSG55	Mangalagi-5	4.00	0.069	0.991	29.60
TSG56	Mangalagi-7	2.96	0.060	0.723	25.30

Table 1. Contd..

Code	Genotypes	100 Seed weight (g)	Phytic acid (mg/g)	IP (mg/g)	Yield/pl (g)
TSG57	Mangalagi-8	2.94	0.078	0.996	35.75
TSG58	Mudbal-3	3.04	0.872	1.118	23.53
TSG59	Nagur	2.60	0.310	1.127	23.50
TSG60	Nalwar-2	2.62	0.060	1.309	41.30
TSG61	Niralkodi	3.26	0.256	1.129	41.20
TSG62	Niralkodi-1	3.96	0.551	1.129	21.10
TSG63	Sugur-1	2.84	0.676	0.410	20.00
TSG64	Sugur-2	3.36	0.140	0.130	14.53
TSG65	Sugur-4	3.06	0.658	0.387	39.40
TSG66	Hadabagatti-2	2.72	0.042	0.128	30.80
TSG67	Soppanapalli	2.88	1.140	0.770	31.90
TSG68	Kaut aurad	3.24	0.827	0.317	59.28
TSG69	Raddewadi	2.68	1.149	0.326	25.10
TSG70	Rawar-1	2.88	0.470	0.245	29.00
TSG71	Sannur	3.30	0.345	0.150	34.05
TSG72	Savalagi-2	3.00	0.104	0.216	2.50
TSG73	Sharnshagi	3.54	1.050	0.195	26.45
TSG74	Tandur-2	3.28	0.988	0.198	55.80
TSG75	Tengalli-1	3.28	1.033	0.270	32.30
TSG76	Tengalli-4	3.16	0.069	0.360	45.53
TSG77	Tengalli-6	4.00	0.818	0.277	76.50
TSG78	Tengalli-7	3.66	0.488	0.092	16.70
TSG79	Tegginalli-9	2.60	0.836	0.274	51.48
TSG80	Tengalli-10 (wh)	2.54	0.149	0.362	34.73
TSG81	Tengalli-10 (Y)	3.28	0.854	0.356	52.15
TSG82	Pop sorghum	2.90	0.095	0.349	13.48
TSG83	M-35-1	4.01	0.542	0.272	28.82
TSG84	Chadachan Mugijola	3.26	0.738	0.297	35.83
TSG85	Savalagi-3	3.06	1.122	0.349	51.93
TSG86	Gundurgthi-2	2.72	0.310	0.425	44.23
TSG87	Tandur-1	3.24	0.212	0.380	21.13
TSG88	Gulbarga-1	2.80	0.783	0.613	33.30
TSG89	Gulbarga-3	3.04	0.658	0.385	47.10
TSG90	Dharwad local-1	2.40	1.550	0.601	45.95
TSG91	Dharwad local-2	2.60	0.301	0.398	32.20
TSG92	Mangalagi-4	3.94	1.211	0.398	46.90
Varieties					
TSG93	DSV-4	2.70	1.675	0.288	15.75
TSG94	Barsi Jowar	3.94	0.631	0.383	42.00
TSG95	DSV-5	3.61	0.212	0.263	47.57
TSG96	SPV-1829	3.38	0.845	0.462	31.25
TSG97	Phule Revathi	3.37	0.256	0.171	30.03
TSG98	Muguthi	3.73	0.345	0.254	38.30
TSG99	GRS-1	3.08	0.747	0.069	27.80
TSG100	CSV-14R	3.10	0.524	0.299	52.00
TSG101	JP-1-5	3.18	0.872	0.482	25.30
TSG102	CSV-22	2.88	0.934	0.338	15.15
TSG103	PC-6	2.88	1.104	0.922	67.00
TSG104	Phule chitra	2.74	0.372	0.883	38.50
TSG105	Phule Maulee	2.94	0.078	1.321	17.80
TSG106	Phule Vasudha	2.44	0.925	0.166	42.30
TSG107	PKV Kranthi	3.06	0.194	0.132	25.50
TSG108	Selection-1	3.90	0.917	0.432	40.30
TSG109	CSV-15	3.04	2.550	0.153	32.98
TSG110	CSV-18R	3.40	1.996	0.236	56.20
TSG111	CSV-216R	2.48	3.210	0.198	28.60
TSG112	CSV-16	2.32	1.434	0.642	46.30

*cultivars from Sudan and ** Nigeria

Table 2. ANOVA for yield and seed 'P' traits in sorghum using general linear model

Sources of variation	d.f	Mean squares Phytic acid	IP	100-Seed weight (g)	Grain Yield (g/plant)
Genotypes	111	2.18**	0.167**	0.351**	387.85**
Replication	1	0.00	0.0003	0.243	9.008
Error	111	0.0051	0.0004	0.051	2.98

* and ** significant at p= 0.05 and 0.01, respectively

Table 3. Univariate analysis and genetic variability components in sorghum landraces/varieties

Traits	Range	Mean	Vg	Vp	GCV	PCV	H^2
Yield (g/plant)	2.5-76.5	31.1	192.4	195.4	44.5	44.8	98.5
100 Seed weightt (g)	2.06-4.1	3.1	0.15	0.20	12.4	14.3	74.7
PAP (mg/g)	0.015-4.45	1.03	1.02	1.05	98.38	99.68	97.43
IP (mg/g)	0.006-1.32	0.39	0.08	0.08	74.2	74.4	99.5

H^2: broad sense heritability; GAM: Genetic advance over mean; Vg and Vp: Genetic and phenotypic variances; GCV, PCV: Genetic and phenotypic coefficient of variation

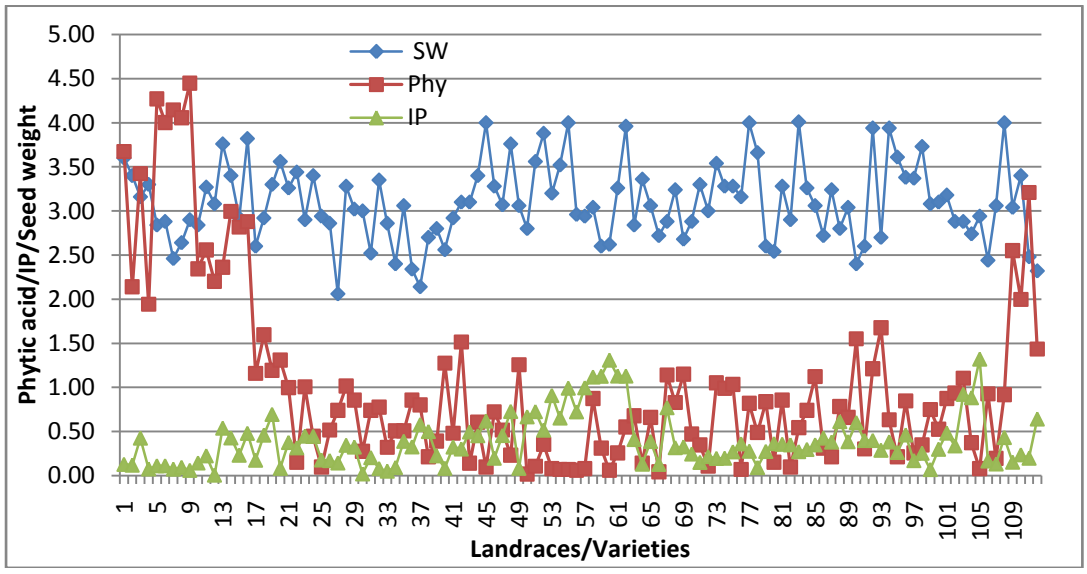

Fig. 1 Variability among the sorghum genotypes for seed weight (SW), phytic acid and IP

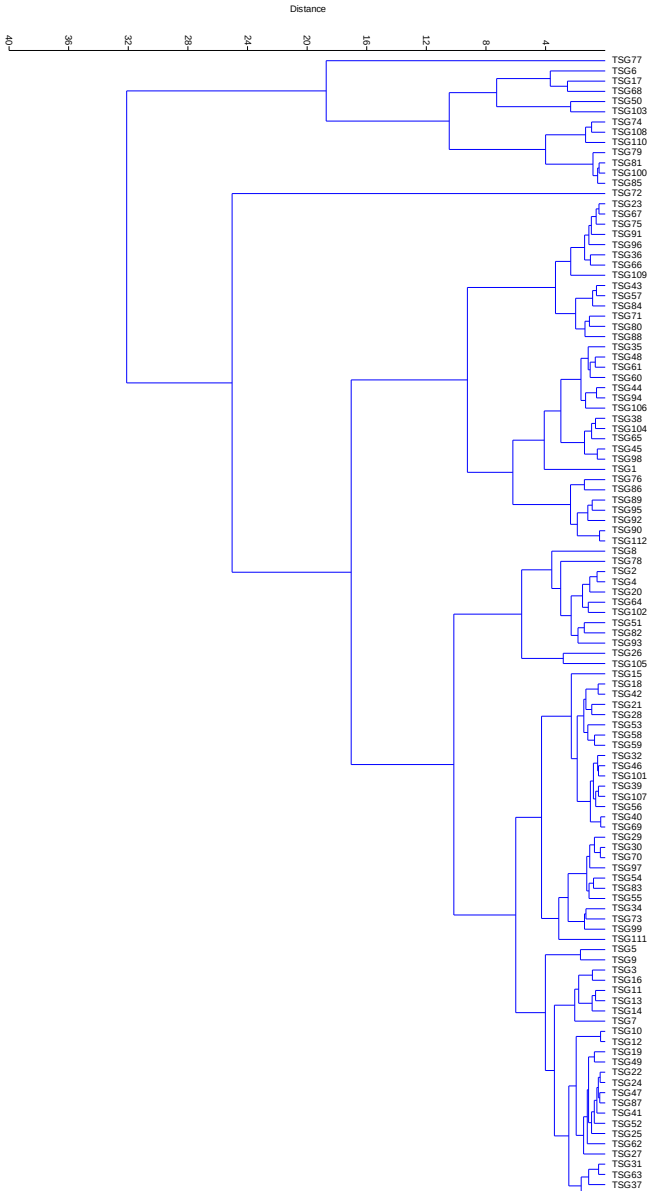

Fig. 2 Dendrgram based on Euclidean distances for seed yield, PAP and IP contents in sorghum genotypes.