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ANTI-NUTRITIONAL FACTORS AND ANTIOXIDANT CAPACITY IN SELECTED GENOTYPES OF SORGHUM [*Sorghum bicolor* L. (Moench)]

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Abstract- Sorghum is a staple food in the arid and semi-arid tropics of Africa and Asia with nutritionally superior grains compared to fine cereals. Nutritional studies in sorghum are mostly confined to major entities like carbohydrate, protein and fat, and very limited reports are available on anti-nutritional factors and antioxidant capacity. With growing interest on better health of the population through diet based interventions for both rich and poor, like functional foods, sorghum will gain prominence in the diets because of its nutritional benefits. Contents of total phenolic compounds, phytates and fibre, and antioxidant capacity (TEAC) were estimated in grains of 200 sorghum genotypes including adapted cultivars and parental lines. Significant variability was observed for all the factors studied. The total polyphenols ranged from 44 to 1272 mg gallic acid eq./100 g, and the highest polyphenols were present in SSG 59-3 followed by Urja (1135-1272mg gallic acid eq./100 g). Phytatecontent varied between 720 and 3909 mg/100g. The maximum phytate was found in the germplasm accession IS 8525 (3909 mg/100g) followed by IS 14131 (3903 mg/100g). Fibre content also exhibited wide range (5.2-20.9%). The highest antioxidant capacity was shown by SSG 59-3 followed by IS 8525 and Urja. Results were confirmed with high repeatability and very high correlation ($r = 0.6\text{-}0.9$, $p < 0.01$). The information generated will aid in identifying suitable donor sources for development of trait-specific sorghum genotypes suitable for end-products that can act as functional foods.

Keywords- Antioxidant capacity, Nutritional quality, Phytate, Polyphenols, Sorghum.

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Introduction

Sorghum is the fifth most important cereal crop by area planted in the world, grown in nearly 100 countries and supporting more than 300 million lives in Africa and Asia. It is an important staple crop in the arid and semi-arid tropics, acting as principal source of energy and other nutrients for millions of the poor people. The tolerance of sorghum to drought and good adaptation to marginal growing conditions makes it a suitable crop for the semi-arid tropics. Sorghum is inexpensive and nutritionally comparable or even superior to major cereals [1].The grains are rich in several phytochemicals and trace minerals because of which, these are now considered as “nutritious grains”. Sorghum is used in many food preparations, the most common forms being boiled grains or ground flour. More than 80% of global sorghum area of 42.12 m ha [2] lies in developing countries, mainly in the African and Asian continents, where sorghum grain is grown primarily for food. India ranks second in terms of area sown to sorghum (6.18 m ha) and grain production (5.28 m tonnes) globally [2]. In India, sorghum is grown both in rainy and post-rainy seasons. The annual per capita consumption by rural consumers in the major sorghum-producing regions is up to 75.2 kg/year [3]. Though over the years the consumption of sorghum is decreasing due to easy availability of rice and wheat, there is a growing awareness among the urban population that sorghum is a health food because of its nutritional superiority, especially higher dietary fibre and antioxidant capacity due to presence of phytochemicals that were earlier known to be anti-nutritional.

Sorghum is a rich source of phytochemicals including tannins and phenolic acids. The anti-nutritional factors present in sorghum grain are mainly polyphenols and phytic acid. Polyphenols are the secondary metabolites produced and they inhibit protein digestibility as they bind the proteins present in grain and make them unavailable for the intestinal absorption. The polyphenols (tannins) and phenolic acids present in sorghum are generally associated with grain pigmentation. Polyphenols also interfere with bio-availability of other major nutrients [4]. But, these phytochemicals have potential to significantly impact human health through high antioxidant activity against different free radicals *invitro* [5]. Sorghums vary widely in their phenolic composition and content, with both genetics and environment affecting the kind and level of phenolic compounds. The phytochemicals have gained increased interest in the recent years due to their antioxidant activity, cholesterol lowering properties and other potential health benefits.

Phytic acid is the primary storage form of phosphorus in cereal and legume seeds, which is deposited as a mixed phytate (myo-inositol hexakisphosphate) salt of minerals such as iron, zinc, calcium or magnesium during seed development [6]. The important nutritional implication of phytic acid is that it interacts with proteins, vitamins, and several minerals, thereby restricting their bio-extractability [7]. Phytic acid also complexes with micronutrients in other foods during intestinal digestion. It is therefore important to reduce phytate content in the seeds to improve micronutrient bioavailability and phosphorus utilisation. Though phytic acid interferes with the iron bioavailability, the same property of iron binding is now considered as positive since excess iron increases oxidative stress.

The term dietary fibre is used to describe a variety of indigestible plant polysaccharides including cellulose, hemicelluloses, pectins, oligosaccharides, gums and various lignified compounds. The major insoluble fibre component of sorghum is reported to be cellulose, which varied from 1.19 to 5.23% in differentcultivars [8]. Dietary fibre has certain adverse effects on the availability of some nutrients, especially iron and zinc. Though sorghum grain has a nutritional profile better than that of rice, the bioavailability of iron and zinc is poor owing to high fibre content compared to other cereals [9].

Studies on nutritional properties of sorghum are more or less limited to major entities like carbohydrates, protein and fat. Very limited reports are available on genetic variability present for factors likepolyphenols, phytates, fibre, etc., as these were considered as anti-nutritional earlier. But, with increasing knowledge about their health promoting attributes, these can now be considered as nutraceuticals. Therefore, a detailed investigation on these factors in sorghum grains and its importance as a source of dietary antioxidants for a large section of population is important. To the best of our knowledge no research effort has been undertaken in case of sorghum with a large number of genotypes to detect variability for polyphenols, phytates, fibre, etc. This paper reports the genetic variability for the above phytochemicals and antioxidant capacity among 200 sorghum genotypes comprising of popular cultivars and parental lines, advance breeding lines and selected germplasm accessions.

Material and method

Plant material

The material for the study consisted of grain samples from popular sorghum cultivars and parental lines (49), advance breeding lines with high yield levels (34) and selected germplasm accessions (117) collected from major sorghum growing states of India [Maharashtra (41), Karnataka (32), Madhya Pradesh (12), Andhra Pradesh (13) and Tamil Nadu (3)] as well as IS (International Sorghum) lines (16). Replicated grain samples collected from each of the genotypes, uniformly dried to a moisture level of 11-12%,were subjected to the estimation of contents of polyphenols, phytate, fibre, and trolox equivalent antioxidant capacity (TEAC).

Total polyphenols

Total polyphenol content in the grains was estimated as per Saucier and Waterhouse [10]. For each analysis, 1.5ml distilled water + 0.1 ml of sample extract (0.1 g/3ml of 70% acetone) + 0.1ml of Folin-Ciocalteau reagent was added and after 30 seconds, 0.3ml of $NaHCO_3$was added. Samples were incubated at room temperature in dark for 2 h. The absorbance of the developed blue colour was read at 700nm. Values were expressed as mg gallic acid eq./100 g (mg GAE/100 g).

Phytate

Powdered sample was extracted in 2.4% HCl for 3 h (0.1g/5 ml). 0.5ml supernatant +0.5ml 2.4% HCl + 0.2 ml $FeCl_3$ (0.00145g/ml 1N HCl) were mixed and boiled for 15 min, immediately cooled. To 1 ml of supernatant, 0.25ml N/2 HCl + 0.5ml 10% KCNS+ 2.5ml N/6 HCl was added. Blood red colour was developed, which was read at 540 nm. The standard phytate was run along with the sample. The values were expressed as mg/100g [11].

Total fibre

For the estimation of total fibre, 100 mg of powdered sample in a conical flask and 25 ml of phosphate buffer (pH 5.9) containing alpha amylase was incubated at 37o C for 3 h, boiled and centrifuged. To the residue, 50 ml of Neutral Detergent solution + 0.1 decalin + 0.25 sodium sulphite was added, refluxed for 2 h, filtered through previously weighed Whatman filter paper, washed with boiled water and then with acetone. Paper was dried overnight and weight was taken. The difference of weight was taken as total fibre [12].

Trolox equivalent antioxidant capacity (TEAC)

This was estimated as per Miller and others [13]. To 1ml 2, 2'Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salts (ABTS) solution 0.01ml of sample extract(0.1g/2.5ml in methanol) was added. The absorbance was taken at 734 nm. Again 0.01ml of sample extract added in same reaction mixture and absorbance was read. The steps were repeated for 5 times. The antioxidant capacity was represented as mg trolox eq./100g (mg TE/100 g).

Data analysis and confirmation

The replication-wise data were analyzed by ANOVA using WINDOSTAT Ver. 7.5 statistical package (www.windostat.org) and results were tabulated category-wise. The variability in each category of genotypes was represented as box plots using software Statistix Ver. 8.1. Thirty-three genotypes, which showed either extreme values for different parameters or important as donors for different agronomic traits were reanalyzed (except fibre) to confirm the results. Results of both the sets were compared factor-wise. A two-sample student's *t*-test was performed to test the equality of the population means that underlie each sample using MS Excel 2007. The *t*-values were plotted against the genotypes to represent the repeatability of the results for different factors. The Pearson correlation was used to determine relationships between the two sets of data as well as among the factors studied.

Results and Discussion

Use of sorghum grains as staple food is on the decline, while new processed and value added food products for human consumption are emerging, which are likely to play a significant role in diversifying sorghum utilization. The health benefits of sorghum based food products are fairly known, still, the extent of research on the nutritional quality as well as its improvement through either breeding or molecular approaches is meager. The knowledge on genetic variability for anti-nutritional factors or antioxidant capacity is essential for overall promotion of sorghum in the diets as health food.

Total polyphenols

Significant variation was observed for polyphenols among all the category of sorghum material studied [Fig-1]. The cultivars and parental lines showed a range of 44-1272 mg GAE/100 g with a mean of 166.3 mg GAE/100 g, while the germplasm accessions showed a slightly lower range of 66-934 mg GAE/100 g with a mean of 137 mg GAE/100 g [Table-1]. The breeding lines tested had a narrow range of 52-205 mg GAE/100 gwith a mean of 91 mg GAE/100 g. Among the cultivars the highest polyphenols were present in SSG 59-3 (1272mg GAE/100 g), followed by Urja (1135mg GAE/100 g), while among the germplasm accessions ELG 8 (IC 568344) (935mg GAE/100 g) had the highest polyphenol. The germplasm accessions collected from Tamil Nadu (EA series) and Maharashtra states (ELG series) had high values of polyphenols while the lowest levels were found in accessions collected from some parts of Karnataka, Maharashtra and Andhra Pradesh (PEC series). The high content of polyphenols in SSG 59-3 and Urja may be due to presence of dark coloured glumes in these genotypes.

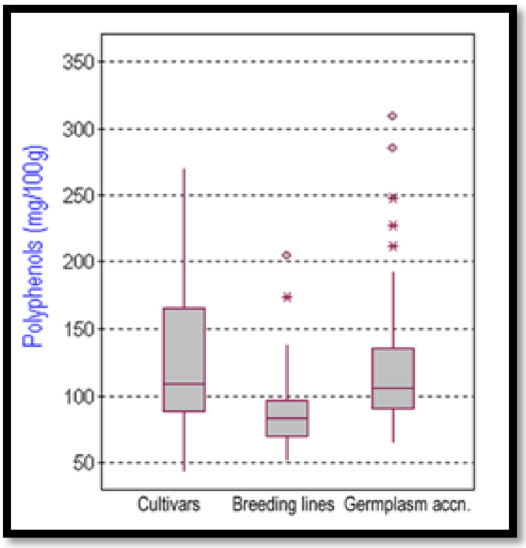

Fig-1 Variation for polyphenol content among sorghum genotypes

Table-1 *Variation for anti-nutritional factors and antioxidant capacity in sorghum genotypes*.

	Polyphenol (mg gallic acid eq./100 g)	Phytate (mg/100 g)	NDF (%)	TEAC (mg troloxeq./100 g)
Cultivars				
Mean	166.3	2410.0	13.3	147.9
Minimum	44.0	719.6	5.2	33.4
Maximum	1272.0	3415.0	20.9	2238.0
C.V. (%)	9.3	5.1	13.9	7.8
C.D. (0.05)	31.2	245.8	3.7	23.1
h^2 (bs)	0.99	0.95	0.66	0.99
Breeding lines				
Mean	91.0	2041.0	12.8	55.3
Minimum	51.8	878.9	7.2	32.8
Maximum	204.8	2994.0	19.6	109.5
C.V. (%)	11.5	5.1	8.5	12.7
C.D. (0.05)	21.3	212.4	2.2	14.3
h^2 (bs)	0.91	0.96	0.91	0.88
Germplasm accessions				
Mean	136.9	2551.0	12.2	114.2
Minimum	65.6	1496.0	6.0	36.7
Maximum	934.3	3909.0	19.7	1907.0
C.V. (%)	12.3	4.2	12.9	29.5
C.D. (0.05)	33.4	211.9	3.1	66.7
h^2 (bs)	0.98	0.91	0.72	0.98

[NDF: Neutral Detergent Fibre; TEAC: Trolox equivalent antioxidant capacity; h^2 (bs): Heritability (broad sense)]

The lowest polyphenols content was found in AKMS 14B (44 mg GAE/100 g), which is the parental line of popular sorghum hybrid CSH 14 in India. Most of the coloured accessions showed high polyphenolic content. But a few light coloured accessions also showed relatively high polyphenolic content *viz.,* GGUB 39 (IC 319882), N 13 and B 58586.

Significant correlations between pericarp colour and sorghum phenols have been reported earlier [14]. A negative correlation between the colour and total phenols was observed suggesting that darker grains contain higher levels of phenolic compounds. It is also reported that sorghums with a pigmented testa increase antioxidant activity [14-15] due to the presence of tannins, which are more potent antioxidants. Correlation between antioxidant activity and tannins (r = 0.79) [15], or antioxidant activity and flavan-4-ol levels (r = 0.88) [14] among non-tannin sorghums with a red pericarp have been reported. Foods which were rich in polyphenol content had higher antioxidant activity [16]. But, as tannins in sorghum grains have been shown to decrease protein digestibility and consumer acceptance, selection in sorghum breeding programmes is mostly restricted to non-tannin types. The lower mean polyphenol content in the breeding lines in the present study testifies this. The heritability (broad sense) (proportion of phenotypic variation that is due to genetic factors) values were found to be very high for this trait in all three category of material [Table-1] indicating lesser influence of environment and hence, greater gains through breeding programmes.

The results of 33 genotypes reanalyzed to confirm the polyphenol contents showed that the values were repeated in 79% of the cases (26/33 genotypes). The student's *t*-test indicated significant difference between the mean values in genotypes like EA 10, IS 8525, SSG 59-3, 27B, IS 2122, 2219B and HC 308 with significant *t*-values [Fig-5]. Very large difference observed in case of SSG 59-3 and IS 8525 is because of the fact that the dark coloured glumes in these genotypes were removed during the reanalysis, which confirms the previous reports on darker grains having higher levels of phenolic compounds [14]. The two sets of values were highly correlated (r = 0.61, $p < 0.01$), while exclusion of genotypes with large differences raised the correlation further (r = 0.87) confirming the repeatability of the result for polyphenol content.

Phytate

Variability for phytate was significant in all three groups of genotypes studied [Fig-2] with advance breeding lines showing slightly lower mean value [Table-1]

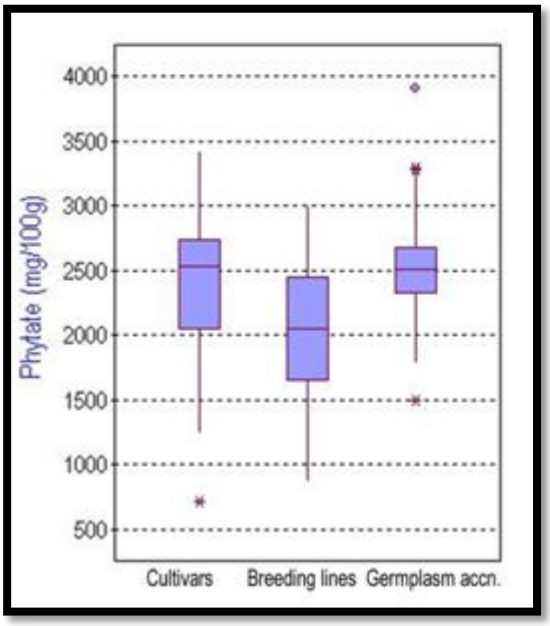

Fig-2. Variation for phytate content among sorghum genotypes

compared to cultivars and parental lines, and germplasm accessions. The range of values among the genotypes was slightly lower in case of advance breeding lines while it was the highest in cultivars and parental lines. Among the cultivars and parental lines the highest phytate content was observed in DJ 6514 (3415 mg/100 g), a variety with very small grain size and high susceptibility to shoot fly, while lowest phytate content was estimated in POP 52 (IC 308676) (719.6 mg/100 g), a germplasm suitable for popping in sorghum. Incidentally, POP 52 has been identified as shoot fly tolerant germplasm, and thusphytate content may also have a role in shoot fly tolerance. Among the germplasm accessions, IS 8525 contained maximum phytate (3909 mg/100 g) followed by IS 14131 (3903 mg/100 g).The IS lines in general had high phytate content, followed by accessions collected from Madhya Pradesh(GGUB series) and Tamil Nadu(EA series). The variability observed in the present study is slightly on the higher side compared to previous reports of 875-2212 mg/100 g [17] and 5.9-11.8 mg/g[18] in sorghum. When the selected 33 genotypes were reanalyzed, the phyta te contents were nearly the same except in case of 13 out of 33 genotypes. Very large differences in mean values were observed in case of IS 8525, IS 18551 and CSV 17 [Fig-5]. The correlation between the two sets of values ($r = 0.85$, $p < 0.01$) was highly significant indicating the repeatability of the trait estimation.

Most studies on phytates concentrated on its mineral-binding capacity which may result in marginal or frank mineral deficiencies in animals and humans [19]. Therefore, a reduced level of phytate in the diet is preferred nutritionally. However, currentlythere is evidence that dietary phytate may have beneficial effects. Positiveeffects against carcinogenesis have been shown with in vitro cell culturesystems, in mice, rats and guinea pigs [19]. Also, phytates appear to provide positive health effects for human populations with optimal micronutrient intakes [20]. Several animal and epidemiological studies demonstrate beneficial effects of dietary phytates including decreased risk of heart disease, renal stone formation, and colon cancer [21]. Hence, nutritionists should work out an optimum level for phytates in sorghum which will have a balance between health benefits and mineral bioavailability.

Dietary fibre

In general high fibre content leads to poor digestibility and availability of nutrients. But, higher dietary fibre may prove beneficial in case of diabetics because of prolonged release of energy leading to a low pressure on insulin [17]. Sorghum is a good source of fibre, mainly the insoluble (86.2%) fibre. Sorghum contains 2.5-9% total dietary fibre [22]. In the present study, the Neutral Detergent Fibre (NDF) ranged between 5.2-20.9% with an average of 13.3% in the cultivars and parental lines [Table-1], [Fig-3]. The highest fibre content was observed in AKR 150, the male parent of popular hybrid CSH 14. This was followed by Pant Chari 5 and SSG 59-3, both forage sorghums. Among the germplasm lines, the NDF ranged from 6.0 to 19.7% with an average of 12.2%. The accessions EP 99 (IC345188; collected from Maharashtra), EA 10 (IC 345252), EA 6 (IC 345248) (collected from Tamil Nadu) and PEC 7 (IC 392130 collected from Maharashtra) recorded NDF above 17%. In the breeding lines the variation for NDF was low with very high heritability value (0.91) [Table-1]. The genotypes identified with high fibre content can act as potential donors for development of improved cultivars with high fibre content, which will specifically be suited for development of high-fibre biscuits and other bakery and food products that are in high demand.

High tannin variety of sorghum was found to have higher level of fibre (9.2%) compared to low tannin variety (7.6%) [23]. Hence, sorghums with a pigmented pericarp are suitable for special food products with high levels of dietary fibre and antioxidants [24]. However, in the present study, there was no significant association ($r = 0.07$-0.18) between the polyphenol content and NDF [Table-2]. In food products where high fibre content is not preferred, decortication of the grain is one of the methods to remove fibre, which can decrease the fibre content by 49–89% [25]. Pearling (partial removal of bran and germ) in sorghum also leads to reduction in crude fibre and thereby enhances the cooking qualities [26].

Table-2 *Relationship between different nutritional factors*

	Genotype	*Phytate*	*TEAC*	*NDF*
Polyphenol	Cultivars	0.18	0.97***	0.18
	Breeding lines	0.41*	0.40*	0.07
	Germplasm	0.36***	0.91***	0.08
Phytate	Cultivars		0.23	0.01
	Breeding lines		0.38*	0.12
	Germplasm		0.31**	0.03
TEAC	Cultivars			0.17
	Breeding lines			0.18
	Germplasm			0.03

[* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NDF: Neutral Detergent Fibre; TEAC: Trolox equivalent antioxidant capacity

Trolox equivalent antioxidant capacity (TEAC)

Measuring the antioxidant capacity is important before promoting sorghum or other millets as functional food grains. In a previous study, among the whole grain cereals sorghum exhibited higher antioxidant activity (24.25%) as compared to other cereals [16].The antioxidant capacity measured as TEAC in the present study showed very wide range both for cultivars and germplasm accessions [Table-1] but some of the extreme values were outliers [Fig-4]. Excluding SSG 59-3 (2238 mg TE/100g) and Urja (1587 mg TE/100g), the values for cultivars ranged between 33.4 and 211.8 mg TE/100g. Among the germplasm accessions, IS 8525 (1907 mg TE/100g) recorded the highest TEAC followed by ELG 8 (IC 568344) (1588 mg TE/100g). The breeding lines in general had low TEAC values [Table-1]. The high antioxidant activity observed in SSG 59-3 or IS 8525 or ELG 8 may be due to the fact that these genotypes have very dark coloured glumes. A few more accessions with red colour *viz.,* EA 10 (IC 345252) (452 mg TE/100 g) and EA 11 (IC 345253) (290mg TE/100 g) also recorded moderately high TEAC. But not all coloured accessions showed higher TEAC values- EP 108 (IC 345197) is reddish orange in colour but had average TEAC of 132mg TE/100g, and EP 107 (IC 345196) which is light brown in colour but had only 143mg TE/100g antioxidant capacity. Similarly, all colourless accessions did not show lower TEAC values. The grain mold resistant B58586 is cream coloured, but had TEAC of 177mg TE/100g, and GGUB38 (IC 319881)is white in colour but had TEAC of around 200mg TE/100g. Also, GGUB34 (IC 319877), GGUB 31 (IC 319874) and EP95 (IC 343594) showed above average TEAC though had white grains. Since all polyphenols are not coloured, there may not be a direct relationship between colour of the grain and antioxidant capacity. The heritability values were very high (0.87-0.99) for this trait [Table-1].

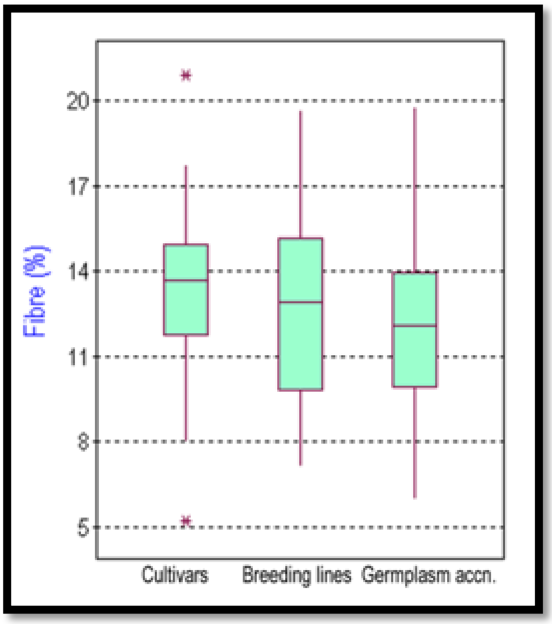

Fig-3. Variation for total fiber content among sorghum genotypes

The values for TEAC in the 33 selected samples reanalyzed were nearly the same as old values except for genotypes like SSG 59-3 and IS 8525, as the coloured glumes were removed before sample preparation. The correlation between the two sets of values was highly significant ($r = 0.92$, $p < 0.001$) indicating the repeatability of the results [Fig-5]. In case of SSG 59-3 the TEAC dropped to 801.6 mg TE/100 g, whereas in case of IS 8525 the TEAC after removing the glumes was 792.4 mg TE/100 g. However, both the genotypes had very high antioxidant capacity in comparison to other genotypes even without the glumes indicating that the values are related to the total polyphenol content in the grains. Higher antioxidant activity of cereals and processed foods which are rich in polyphenol content has previously been reported [16]. The correlation between the polyphenol and TEAC in the 33 samples was highly significant ($r = 0.65$, $p < 0.001$) similar to the corresponding old value ($r = 0.97$, $p < 0.001$) though lower in

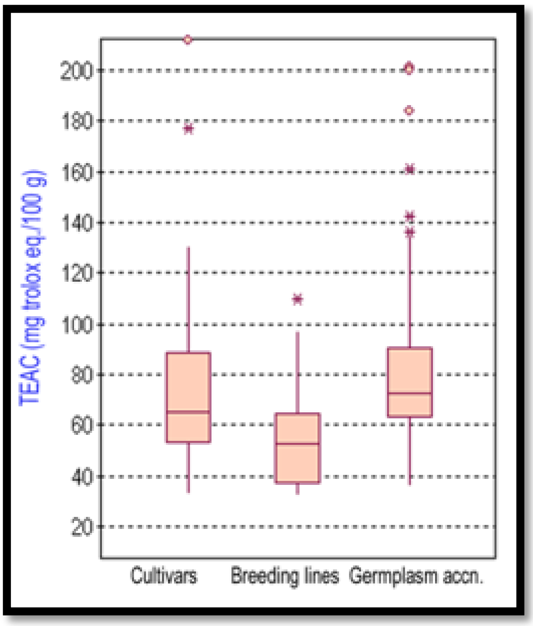

Fig-4. Variation for TEAC among sorghum genotypes

magnitude. Partial or complete removal of seed coat in sorghum can result in lower polyphenols and antioxidant activity, if needed, as observed in case of SSG 59-3 or IS 8525 when glumes were removed.

Association between different nutritional factors

A strong to very strong association between polyphenol and phytate content was observed among the breeding lines and germplasm accessions [Table-2]. Similarly, the antioxidant activity of the genotypes was found to be strongly associated with polyphenol and phytate content. The correlation between total polyphenols and TEAC was highly significant in case of cultivars (r = 0.97) and germplasm accessions (r = 0.91) but in case of breeding lines the magnitude was moderate (r = 0.4, p =0.02). Association of antioxidant activity with polyphenol content in sorghum has been well reported [14-16]. The TEAC in the cultivars and parental lines was found to be less related to the phytate content as correlation coefficient was non-significant. Relationship of fibre with other factors was not significant.

Based on the results obtained for the different factors, the sorghum genotypes irrespective of the category were ranked depending upon the antioxidant quality and nutritional superiority [Table-3].

In case of polyphenols, phytate and TEAC, genotypes with high mean values were given top ranks; whereas for fibre content, genotypes with least values were accorded top ranks. Though a single best genotype could not be identified across the factors, some of the top ranked genotypes like Urja, ELG 8, EA 10, GGUB 34, etc., can act as potential donors in specific breeding programmes targeted at end-product specific varietal or parental line development. Sorghum bred specifically to produce high levels of different types of phenols can also be portrayed as an antioxidant rich health-promoting staple for the health-conscious elite consumers.

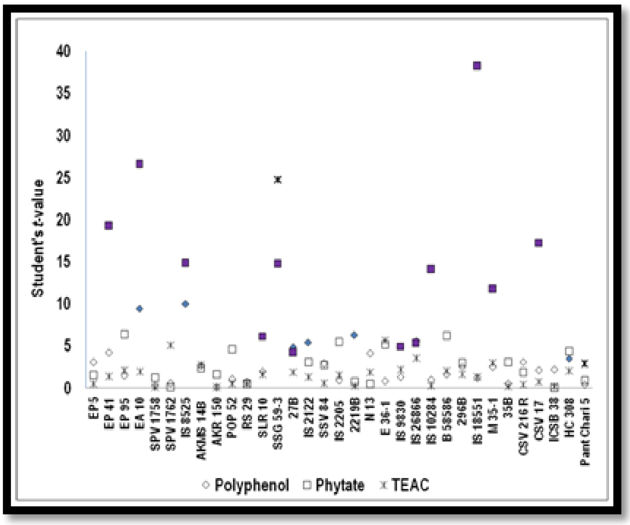

Fig-5. Plotting of student's t-value against the genotypes (solid fills indicate significant t-values)

Conclusions

Considerable genetic variability has been observed for contents of polyphenols, phytate, fibre and antioxidant activity in the sorghum genotypes tested. The repeatability of results could be established with a representative sample. Sorghum genotypes identified according to antioxidant quality in the desirable direction can find place in special breeding programmes, which in turn can support the ongoing food processing and value-addition efforts in sorghum. It should be possible to utilize the variation identified in sorghum breeding programmes aimed at development of improved cultivars endowed with high yield potential along with desired levels of nutritional factors.

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Table-3. *Ranking of sorghum genotypes according to antioxidant quality indices.*

Rank	Polyphenols (mg gallic acid eq./100 g)	Phytate (mg/100 g)	NDF (–) (%)	TEAC (mg trolox eq./100 g)
1	SSG 59-3	IS 8525	SLR 10	SSG 59-3
2	Urja	IS 14131	EP 6	IS 8525
3	ELG 8	DJ 6514	IS 10284	ELG 8
4	IS 8525	E 36-1	EP 93	Urja
5	EA 10	GGUB 34	SPV 1799	EA 10
6	EP 108	EP 115	SPV 1809	EA 11
7	EA 11	EP 108	EP 5	GGUB 34
8	GGUB 34	IS 10284	IS 8525	N 13
9	CSV 15	EP 68	SPV 1761	EP 33
10	EP 107	CSV 19SS	EP 138	GGUB 38
11	N 13	EA 10	SPV 1764	GGUB 31
12	CSV 17	IS 18551	IMS 9B	B 58586
13	GGUB 39	IS 2312	IS 2312	EP 95

14	B 58586	IS 9830	EL 80	EP 107
15	EP 95	EL 80	EP 48	GGUB 37
16	SPV 1805	SSG 59-3	EP 37	EC 34
17	GGUB 38	GGUB 1	SPV 1757	SEVS 3
18	CSV 18	SPV 1792	104B	EP 108
19	CSV 14R	RS 673	SEVS 3	AKMS 14B
20	35B	GGUB 33	EP 11	AKR 150

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