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GT-biplot analysis of some biochemical characteristics and mineral composition of different sorghum (*Sorghum bicolor* L.) sprouts

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Abstract

Background and Objectives: The nutritional value of the sprouts has gained interest in recent years because of supplying to fiber, vitamins, minerals, and bioactive compounds such as antioxidants into the diet. The aim of the present study was to reveal the nutritional characteristics of the different sorghum genotypes exposed to sprouting process. For this purpose, some biochemical characteristics and basic mineral profiles of the sorghum sprouts were investigated.

Findings: According to the results, protein content ranged between 12.17% and 32.24% while the lowest total starch content was determined as to be 3.37% and the highest was 32.71%. Besides, dietary fiber content was in the range of 30.27%–46.42%. Total phenolic content and antiradical activity values of the sprouts were determined as in the range of 3.61–8.42 mg GAE/g and 11.63%–19.51%, respectively.

Conclusion: The results showed that the sprouting process improved the nutritional quality of sorghum compared to grains and also significant variation was observed among the genotypes in terms of examined properties.

Significance and Novelty: The results showed that the nutritional parameters especially phytic acid levels of the sorghum grains could be decreased by sprouting process.

KEYWORDS

bioactivity, classification, nutritional value, sorghum, sprout

1 | INTRODUCTION

Utilization of pulses and cereals is an emerging trend to develop functional foods, depending on nutritional compositions (Medhe et al., 2019). One simple and effective utilization approach is induction of sprouting in seeds (Ampofo & Ngadi, 2020). Germination of seeds is a method to produce sprouts having a high nutritive value

while showing quite low anti-nutritional factors (Mun et al., 2020). Additionally, germination is an effective and low-cost way to improve the bioactive compound contents of the sprouts (Xue et al., 2016). Researchers reported that the free amino acids, minerals, dietary fiber content, total phenolic content, and antioxidant capacity of grains can be improved while reducing the fat and total carbohydrate (Van Hung et al., 2020). Furthermore,

it was informed that the germinated edible seeds showed important bioactive properties such as antidiabetic, anticancer, antiproliferative, antistress, ACE-inhibitory, anti-inflammatory, and neuro-protective effects (Gan et al., 2017). On the other hand, sprouts may have some risks in terms of food safety. Researchers have also studied to find an appropriate action to eliminate or control microorganisms especially pathogens found on sprouts (Zhang et al., 2019).

The agronomic advantages of sorghum (*Sorghum bicolor* L.) such as drought tolerance, high yield, low production cost, and also its high nutritive properties have been provided a popularity for its use in human consumption worldwide (Pinheiro et al., 2021). Sorghum is the fifth most important cereal in the world (Abdelhalim et al., 2021) and is generally used as grain and flour in daily diet (Pinheiro et al., 2021).

Several scientific reports have been informed that the species and age of the seed, stage of seedling growth, germination conditions, biotic stress, and dormancy state specify the chemical composition and bioactive characteristics of the manufactured sprouts (Cid-Gallegos et al., 2020). Also, the germination performance and phytochemical profile of the sprouts in any plant species, may depend on the seed genotype (Falcinelli et al., 2017). Considering all of this information, it was aimed to investigate the nutritive properties and also major mineral profile of the different sorghum genotypes and compare with standard cultivars. This study is one of the reports used many genotypes and aimed to show the effect of genotype variety on the biochemical composition and mineral profile of the sprouts.

2 | MATERIALS AND METHODS

2.1 | Materials

Sorghum seeds were grown in the experimental fields of Erciyes University Agricultural Faculty by using augmented experimental design. Each plot had 70 × 15 cm wide, 5 m long with four rows. According to the soil analysis results, 12 kg/da P₂O₅ and 20 kg/da nitrogen were used for the soil. The whole of phosphorus fertilizer and half of the nitrogen fertilizer were incorporated into the soil with planting, and the other half of the nitrogen fertilizer was given when the plants reached to 30–40 cm tall. During the development of the plants, hoeing was done twice, and weeding was done once. Plants were irrigated with a drip irrigation system after they reached 30–40 cm once a week to eliminate the lack of useful water by determining the field capacity. During the flowering period, seeds were reproduced daily by selfing

for each genotype. Genotypes showing high grain yield were selected to be used in the sprouting process.

2.2 | Production of sorghum sprouts

A total of 36 sorghum genotypes were used as the plant material in the current study. Of these samples, 33 of the samples were sorghum genotypes and the other three samples were standard cultivars (Akdarı, Beydarı, and Öğretmenoğlu). Initially, some impurities were removed from the sorghum seeds. Water-supplemented seeds were then also supplemented with 1.5% of NaOCl (sodium hypochlorite, v/v) and kept in this solution for 45 min. Then, the samples were washed through tap water to remove NaOCl. The seeds were then placed into jars, supplemented with distilled water, kept at 25°C for 24 h. The seeds were subjected to germination process in between filter papers in a climate cabin at 25°C and 85% relative humidity for 7 days. Throughout the process of germination, distilled water was sprayed over filter papers to keep the seeds moist. For analyses, sprouts were removed from the germination ambient at the end of 7th day. Removed sprouts were dried at 55°C for 48 h and the dried samples were ground, passed through 0.5 mm sieve and used in relevant analyses.

2.3 | Biochemical analyses

2.3.1 | Analysis of crude protein content

Sample nitrogen contents were determined by Kjeldahl method and the resultant value was multiplied by a coefficient of 5.7 to calculate crude protein contents of the samples (AOAC, 1990).

2.3.2 | Analysis of condensed tannin content

About 0.01 g of powdered sprout sample was supplemented with 6 ml of tannin solution and boiled in a water bath for 1 h. Following the boiling process, 3 ml of sample was taken, and reading was performed in a spectrophotometer at 550 nm wavelength (Makkar et al., 1995).

2.3.3 | Analysis of phytic acid content

Megazyme Phytic Acid Kit (K-PHYT) was used to determine the phytic acid content of the samples without a purification requirement. One gram of the powdered

sample was digested with 20 ml of HCl (0.66 M) in 50 ml Falcon tubes, and placed in a mixer overnight at room temperature. After digestion, 1 ml of the extract was transferred into a 1.5 ml Eppendorf tube and centrifuged at 13,000 rpm for 10 min. Immediately, 0.5 ml of the resulting extract supernatant was transferred into a new tube for neutralization by the addition of 0.5 ml of NaOH (0.75 M) solution. Neutralized sample was used for enzymatic dephosphorylation reaction. After this step, phosphorus was determined colorimetrically and phytic acid values were calculated using with Mega-Calc software (Kaplan et al., 2019).

2.3.4 | Analysis of total, resistant and nonresistant starch content

Megazyme Resistant Starch Assay (K-RSTAR, Megazyme International Ireland Ltd, Co.) Kits developed based on AOAC (2002). 02 and Zhou et al. (2013) methods were used to determine resistant starch contents of the sprouted sorghum samples. This method is based on removal of nonresistant starch. An aliquot of sample material (100 mg) was combined with sodium maleate buffer (pH 6.0) containing pancreatic α -amylase and amyloglucosidase. Sample was mixed and incubated in a shaking water bath for 16 h at 37°C, to form D-glucose by the combined action of the two enzymes. The reaction was stopped by adding ethanol (99% v/v). The resulting mixture was then washed twice by suspension in aqueous ethanol (50% v/v), followed by centrifugation at 3000 rpm for 10 min. Free liquid was removed, and the pellet was dissolved in 2 M KOH in an ice/water bath by vigorously stirring for 20 min. The solution was neutralized with sodium acetate buffer, and the starch was hydrolyzed to glucose with amyloglucosidase. The samples were centrifuged, and an aliquot was incubated with glucose oxidase/peroxidase reagent (GOPOD) and incubated at 50°C for 20 min. Finally, the absorbance of each solution against the blank was measured at 510 nm for measurement of the resistant starch content. Total starch values were separately obtained by spectrophotometrically (Shimadzu Uv-1800). Then, resistant starch was subtracted from total starch to get non-resistant starch (Amaral et al., 2016).

2.3.5 | Analysis of dietary fiber content

Dietary fiber content was determined in accordance with AOAC 991.43 (AOAC, 1992) method. In this analysis, samples were subjected to series of enzymatic reactions with thermostable α -amylase (100°C,

35 min), amyloglucosidase (60°C, 30 min), and protease (60°C, 30 min) to remove starch and protein from the samples. Enzyme-resistant starch (ERS) was precipitated with ethanol. Samples were then filtered through perforated base of Gooch crucible (Gooch crucible, pore size 40–100 μ m, porosity 2), washed through ethanol (at 78% and 95% concentration) and dried overnight in an oven at 105°C and total dietary fiber level was calculated using mass balance.

2.3.6 | Analysis of major mineral composition

To determine B, Cu, Fe, Mg, Mn, Na, P, and Zn minerals, samples were subjected to microwave digestion with nitric acid – hydrogen peroxide (2:3) in three different steps (first step: at 145°C, 75% microwave power for 5 min; 2nd step: at 180°C, 90% microwave power for 10 min; third step: at 100°C, 40% microwave power for 10 min) in a microwave wet digestion unit (speed wave MWS-2 Berghof products + Instruments Harresstr.1. 72800 Enien Germany) resistant to 40 bar pressure (Mertens, 2005), then readings were recorded in an ICP-OES spectrophotometer (Inductively Couple Plasma spectrophotometer) (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794) (Mertens, 2005).

2.3.7 | Analysis of total phenolic content

For the total phenolic content, the sorghum samples (0.5 g) were extracted with 25 ml of 1% HCl/methanol v/v for 2 h at room temperature. Resultant extract was filtered, and 0.1 ml was supplemented with 1.1 ml distilled water, then with 0.4 ml of Folin–Ciocalteu reagent (diluted 1/10 with distilled water) and 0.9 ml of 0.5 m ethanolamine. Sample tubes were incubated at room temperature for 20 min. Sample absorbance was measured by using a spectrophotometer (Shimadzu Uv-1800, Japan) at 600 nm. Total phenolic content was calculated using the gallic acid calibration curve and the results were expressed in mg gallic acid equivalent (GAE)/g sample (Dykes et al., 2005).

2.3.8 | Analysis of antiradical activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) method was used to determine antiradical activity of the samples. Sample extractions were performed in a shaking water bath with 70% acetone for 2 h at room temperature. Resultant extracts were centrifuged at 2790 g for 15 min and the supernatant was used for the analysis.

Samples were placed in a dark place at -20°C to prevent oxidation. DPPH solution was also kept in the dark conditions. About $150\ \mu\text{l}$ extract was supplemented with $2850\ \mu\text{l}$ DPPH solution and resultant mixture was vortexed and incubated for 30 min. Then, absorbance readings were performed by using a spectrophotometer (Shimadzu Uv-1800, Japan) at 517 nm (Awika & Rooney, 2004). The antiradical activity of the samples was calculated as %inhibition by the following equation (Equation 1)

$$\% \text{ Inhibition} = [(A_c - A_s) \times 100] / A_c, \quad (1)$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample.

2.4 | Statistical analysis

Experimental data were subjected to variance analysis with Statistical Analysis Software 9.0 (SAS Institute). Analyses of variance of the biochemical composition of sorghum landraces was achieved using a least significant difference (LSD) and multiple comparison procedure with a 1% significance level. To determine which genotype or genotypes were the best in terms of which nutrient content, to determine the genotypes to be used in breeding studies and to develop to increase the nutrient content and quality in sorghum, genotypes biplot graphics were generated (Yan, 2014). Biplot analysis was performed using the Genstat 12.0 statistical software. All analysis were repeated two times with three replications.

3 | RESULTS AND DISCUSSION

3.1 | Changes of nutritive parameters of the sprouted sorghum genotypes

The genotype codes of the sorghum samples characterized are given in Table 1. Table S1 shows the biochemical characteristics (dietary fiber, resistant starch, non-resistant starch, total starch, condensed tannin, crude protein, and phytic acid content) of sorghum sprouts. There were highly significant differences among all traits of the genotypes ($p \leq .01$). The lowest dietary fiber content was determined for genotype 11 (30.27%) and the greatest value was for the Akdari genotype (46.42%). Total dietary fiber content of the sorghum flour was determined as to be $6.79 \pm 0.02\%$ in our previous research (Kaplan et al., 2020). It was concluded that the sprouting process provided a significant increment in the dietary fiber content of the sorghum seed, and it could be said that the sprouting process caused a tenfold higher increase in the total dietary fiber content compared to sorghum flour. Bader Ul Ain et al. (2019) reported that the crude fiber contents of two sorghum varieties were determined as to be 3.6% and 4.1%. Dietary fibers are defined as the edible part of plants or analogous carbohydrates, which resist to the hydrolysis by alimentary tract enzymes (Dietary Fiber Definition Committee of the American Association of Cereal Chemists, 2001). Dietary fiber is an important component in maintenance of the functional integrity of the gastrointestinal tract (Zieliński et al., 2005) and beneficial against a variety of diseases, including colon cancer and diabetes (Qiu et al., 2017). Taking into accounts of all this information,

TABLE 1 The codes of genotypes used in the study

Code	Genotypes	Code	Genotypes	Code	Genotypes
G1	PI 167264 03/5	G13	PI 170784 02/3	G25	IS 12850
G2	PI 179051 03/2	G14	PI 174382 02/2	G26	IS 12855/2
G3	PI 179052 03	G15	PI 177077 03	G27	IS 12817
G4	PI 177156 02/4	G16	PI 177077 03/3	G28	IS 41744
G5	PI 170780 03/5	G17	PI 255743 03/3	G29	IS 41739
G6	PI 170780 03/6	G18	PI 167014 03/1	G30	IS 2885
G7	PI 182301 02	G19	PI 167014 03/2	G31	IS 12859
G8	PI 170787 02/2	G20	PI 175919 02/2	G32	IS 21863
G9	PI 166979 03/1	G21	PI 170783 04/2	G33	IS 13211
G10	PI 166967 05/1	G22	IS 12828/1	G34	Akdari
G11	PI 177160 02/5	G23	IS 21864/1	G35	Beydari
G12	PI 170784 02/2	G24	IS 12819/1	G36	Öğretmenoğlu

it could be said that the sorghum sprouts could be evaluated as a food ingredient or substance having more functional properties for gastrointestinal system compared to sorghum flour.

The starch content of all sorghum sprouts was determined and the resistance of the starch against enzyme digestion was also characterized. So, resistance starch and nonresistance starch contents of the starch molecules were also measured (Table S1). The greatest resistant starch (2.16%), nonresistant starch (30.55%), and total starch (32.71%) contents were determined for the genotype 24 and it was seen that this genotype showed significantly higher resistant starch content compared to other sorghum varieties. The lowest resistant starch content was measured for genotype 15 (0.05%) while the lowest nonresistant starch (3.25%) and total starch content (3.37%) were determined for genotype 6. Chen et al. (2019) investigated the total starch levels of 634 sorghum accessions, and they reported that the total starch concentrations were in the range of 60.28%–74.03%. In our previous research, it was also determined that the total starch content of sorghum grains was in the range of 15.42%–85.54% while the resistant and nonresistant starch contents ranged between 1.10%–34.23% and 10.79%–79.61%, respectively (Kaplan et al., 2020). It is clear from these results that the resistant, nonresistant, and also total starch concentrations of the sorghum seeds tended to decrease by the sprouting process with the accordance of previous reports (Singh et al., 2019; Pinkaew et al. 2016). Starch is the storage form of energy and during germination; it is degraded to small dextrin and sugars (Singh et al., 2019). This degradation of starch is attributed to the hydrolytic activity of the amylase enzyme (Sharma & Gujral, 2020).

Crude protein levels of the sorghum sprouts varied between 12.17%–32.24% and the lowest value was obtained for genotype 14 and the greatest protein level was for genotype 3. The protein content of the sorghum grains was reported as in the range of 6.67%–14.33% and the mean value was 10.47% (Kaplan et al., 2020). Badigannavar et al. (2016) also measured the protein content of 112 local landraces and varieties of sorghum as in the range of 3.50%–12.60% as like the results reported in our previous work (Kaplan et al., 2020). It could be certainly said that the sprouting process provided a significant increment in the protein content of sorghum grain structure as reported by the similar previous reports (Devi et al., 2015; Mehta et al., 2007; Obizoba & Atii, 1991). Obizoba and Atii (1991) informed that the breakdown of tannin-protein complexes might be caused to release of free amino acids and also result of protein synthesis. It was also informed that the protein content depends on the balance between protein degradation and protein biosynthesis during germination (Benincasa et al., 2019).

Condensed tannin contents of the sorghum genotypes ranged between 0.80% and 3.39%. The lowest value was

determined for genotype 13 and while the highest condensed tannin level was for Öğretmenoğlu cultivar. Mean condensed tannin content was determined as to be 1.46%. The tannin content in sorghum varieties showed a significant variation ($p < .05$) and it caused a sorghum classification as Type I, II, and III. Type III sorghum may contain up to 50.2 mg tannin level per gram (Xiong et al., 2019). In the current study, only one sample (Öğretmenoğlu cultivar) had condensed tannin concentration as higher than 3%. Hydrophobic association of tannins with seed protein and enzymes was the main reason behind reduction in tannins along with the loss of tannins due to leaching process in water during germination.

The lowest phytic acid content was measured for genotype 28 (0.02%) and the greatest value was for genotype 31 (0.82%). The mean phytic acid levels (0.32%) of the sorghum sprouts were in accordance with the results reported by Mohamed Nour et al. (2010) who informed that the phytic acid contents of Abu Ragaba, Abu Kunjara, and Wad Ahmed cultivars were 206.2, 311.7, and 199.6 mg/100 g, respectively. Additionally, in our previous published paper, the phytic acid content of the sorghum grain was determined as to be in the range of 0.37%–4.09% (Kaplan et al., 2020). According to these results about the phytic acid levels of the sorghum grains, it could be said that germination process reduced the phytic acid values of the sorghum grains. As is known, phytic acid having highly charged six phosphate groups causes the formation of insoluble complexes with mineral cations and proteins that reduce bioavailability and this is big disadvantages for the grains having high phytic acid levels (Duodu et al., 2003). Afify et al. (2011) reported that the germination process has an important effect on the enzymes present in the grains and it activates the endogenous grain phytase enzyme and so the phytate can be broken to myo-inositol phosphate which has no negative effect on the bioavailability of the minerals.

The bioactive properties determined with total phenolic content and DPPH antiradical activity methods were illustrated in Figure 1. The lowest total phenolic content (3.61 mg GAE/g) and antiradical activity (11.63%) were recorded for genotype 13, while the greatest total phenolic content (8.42 mg GAE/g) was obtained for genotype 4 and the greatest antiradical activity (19.51%) was obtained from the genotype 22. Mean total phenolic content was monitored as to be 5.46 mg GAE/g while the mean antiradical activity was 14.92%. Total phenolic content results for the sorghum sprouts were higher than those of Hithamani and Srinivasan (2014) who reported that the total phenolic content of sorghum sprouts was 1.18 ± 0.16 mg GAE/g. Total phenolic content of the sorghum grains varied between 0.19 and 5.06 mg GAE/g and antiradical activity values were in the range of 3.72%–91.48% (Kaplan et al., 2020). Besides, mean antiradical activity of sorghum grains was reported as to

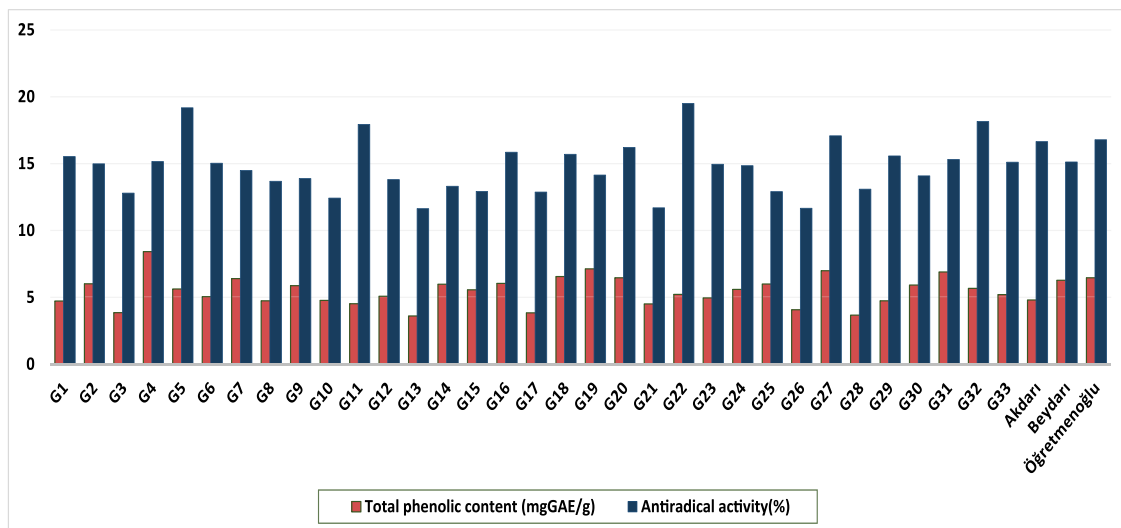


FIGURE 1 Bioactive properties of sorghum sprouts (LSD for TP: 0.31 and LSD for AA: 0.65, Sg. Dg., significant degree; $**p \leq .01$). [Color figure can be viewed at wileyonlinelibrary.com]

be 42.02% (Kaplan et al., 2020). According to the results, it could not be said that the antiradical activity values increased during sprouting. Limmongkon et al. (2017) informed that the phenolic content and antioxidant activity values at each germination stage varied, and antioxidant activity values decreased day by day for some genotypes.

3.2 | Changes in mineral contents of the sprouted sorghum genotypes

Maximum and minimum values of the mineral contents for the sorghum sprouts produced from different genotypes are tabulated in Table S2. There were significant differences in all mineral contents of the genotypes ($p \leq .01$). The data obtained indicated that phosphorus and magnesium were the major mineral constituents of the sprouts. The lowest Cu content (2.17 mg/kg) was obtained for genotype 22 and the greatest Cu content (8.61 mg/kg) was obtained from genotype 9. Mg contents of the sorghum sprouts varied between 222.4 and 1000.2 mg/kg with the lowest value was for genotype 16 and the greatest value was for genotype 28. The lowest Mn content (10.81 mg/kg) was obtained for the Ögretmenoglu genotype, and the greatest value (25.93 mg/kg) was measured for genotype 30. Na contents of the sorghum sprouts were in the range of 106.5 mg/kg (genotype 16) - 430.2 mg/kg (genotype 33). Similar copper, manganese, sodium, and magnesium content results were determined by Afify et al. (2012) for germinated three sorghum varieties. The lowest P content (1543.9 mg/kg) was obtained for genotype 23

and the greatest P content (3242.7 mg/kg) was obtained for genotype 33. The lowest Fe content (44.86 mg/kg) was obtained for genotype 25 and the greatest value (147.2 mg/kg) was for genotype 2. The lowest Zn content was obtained for genotype 9 (22.54 mg/kg) and the greatest Zn content was measured for genotype 6 (93.40 mg/kg). The values belonging to Zn, Fe, and P minerals were found to be higher than the report of Afify et al. (2011) who found as to be 203–275 mg/100 g for P, 3.4–4.71 mg/100 g for Fe and 3.12–3.45 mg/100 g for Zn contents of sorghum sprouts.

Shegro et al. (2012) informed that sorghum grain is a valuable source of K, Ca, Mg, P, Fe, and Zn in diets of Ethiopians, especially for people living under poor socioeconomic situations. The authors investigated the mineral composition of 31 sorghum landrace accessions and grain mineral results were in accordance with our results belong to sprouts except Na (11.5–54.38 mg/kg) and Zn (13.5–34.67 mg/kg). Sprouts had higher Na and Zn content compared to grains. Similar sodium increment was observed by Jan et al. (2018) for germinated *Chenopodium* flour and the authors stated that sodium has an important role in enzyme operations, osmoregulation, muscle contraction, and fluid maintenance.

3.3 | Classification of sprouted sorghum genotypes

The genotype-trait (GT) biplot graph generated for visual assessment of nutritional attributes of sorghum sprouts based on genotypes is presented in Figure 2. The furthest genotypes from the origin of biplot were connected to

in terms of these traits. The third trait group was composed of CP and DF traits and G6 and G12 genotypes were found to be prominent in terms of these traits. PA traits were separated from the other traits and each one constituted a single group. The diagonal genotypes G26 and G13 had superior characteristics in terms of PA. The distance of traits from the center (vector lengths) improved efficiency in assessment of genotypes. The greatest vector lengths were observed in TT, NRS, CT, and AA traits (Figure 2).

The GT biplot graph generated to assess the sprout mineral contents of the genotypes and to see which genotype is prominent for which minerals is presented in Figure 3. There were seven sectors on the graph based on genotype–mineral relationships. Minerals were placed into four sectors. The first sector included Cu and genotype G9 was the diagonal genotypes. The second sector included Fe, Mg, and P with positive correlations among them and the diagonal genotypes G2 were superior to the other genotypes in terms of these minerals. The third sector included Na and Mn and genotypes G30, G33, and G7 were the diagonal genotypes. The fourth sector included B and Zn with positive correlations among them and the diagonal genotypes G6, G20 and G31 were superior to the other genotypes in terms of these minerals.

The second sector included Ca, P, Mg, S, and Mn with positive correlations among them and the diagonal genotypes 19, 24, and 36 were superior to the other genotypes in terms of these minerals. The third sector included Na and Zn and genotypes 2 and 3 were the diagonal genotypes. K alone constituted a single group and there was no diagonal genotype for K. The genotype G9 was placed at the furthest position from the origin. For distances from the origin, this genotype was followed by genotypes G2, G7, G30, G33, G31, G20, G29, and G16. This group of genotypes was clearly separated from the other genotypes and had the greatest values for all minerals. The furthest minerals from the origin (the greatest vector lengths) were identified as B, Zn, Na, Fe, and Cu. These minerals allowed more efficient selection of genotypes as compared to the other minerals (Figure 3).

4 | CONCLUSION

In the present study, genotype effects on the nutritional and mineral characteristics of sorghum sprouts were investigated and the prominent genotypes were determined with GT biplot analysis. As a result of sprouting process, total protein and dietary fiber contents increased while phytic acid and condensed tannins contents

decreased. G22 had superior characteristics in terms of TP and AA while G6 and G12 genotypes were found to be prominent in terms of CP and DF. Sprouts have superior health benefits compared to grains and this study is the first study used a big genetic sorghum resource. As a result of the statistical GT biplot technique, prominent genotypes could be used in further breeding studies.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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