



Biochemical composition of selected lines from sorghum (*Sorghum bicolor* L.) landraces

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Abstract

Main conclusion Present findings revealed a great variation in protein, oil, starch, tannin and phytic acid-like biochemical attributes of sorghum genotypes. GT bi-plot analysis was used for assessment of biochemical analysis results.

Abstract Sorghum is quite resistant to negative environmental conditions. Thus, it has become the basic source of nutrient in majority of developing countries. It is also used as a supporting product against several diseases. In this study, biochemical composition of new 154 sorghum lines, selected from Turkish sorghum landraces through morphological, biochemical and molecular studies, was determined. Present findings revealed based on average of two years that crude protein content of 80 lines (> 9.65%), digestible protein content of 94 lines (> 56.25%), crude oil content of 75 lines (> 3.87%), resistant starch ratio of 32 lines (> 3.93%), starch content of two lines (> 77.07%) and amylose content of 10 lines (> %25.26) were greater than standard cultivar with the greatest values and amylopectin contents of 74 lines (< 47.75%) were lower than the standard cultivar with the lowest values. As the average of years, phytic acid contents and condense tannins contents of the lines, respectively, varied between 0.02 (IS 12850/1)—6.49% (PI 177161 03/2) and 0.09 (IS 12819/1)—5.38% (PI 255738 02/3). With this study, several sorghum lines superior than the standard cultivars were identified. Those lines can be used for direct cultivar registration in short run and can be used as parent materials in further breeding studies to be conducted for various purposes in long run.

Keywords Sorghum · New lines · Protein · Starch · Phytic acid · GT Biplot

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Introduction

Sorghum is an important cereal crop in Asia, Africa and the other semi-arid regions of the world (Afify et al. 2011). It is the fifth common cereal crop worldwide. It constitutes a great source of nutrient (protein, starch and, etc.) and bioactive compounds for humans (de Morais Cardoso et al. 2017). Sorghum is used in cereals, cookies, bread, cakes, flakes, pancakes, couscous, alcoholic and non-alcoholic beverages (Yousif et al. 2012). Its popularity as a functional food is also continuously increasing worldwide (United States Grains Council 2001). Such an increase is not only because of increasing sorghum-consuming population, but also because of developing industrial uses of sorghum (Akintayo and Sedgo 2001).

Starch is a significant component of cereal grains (Wong et al. 2009). Diseases like diabetes and obesity are closely related to carbohydrate consumption (Bray and Popkin

2014). As compared to maize and the other cereals, sorghum grains have lower starch contents and protein digestibility levels (Ezeogu et al. 2005). Such a case then results in different outcomes for human and animal nutrition. Product functionality is directly related to biochemical structure of sorghum (Kaufman et al. 2017). Different amylose contents may alter thermal characteristics of starch (Jane et al. 1999). On the other hand, molecular organization of amylopectin plays a significant role in functionality of starch.

Resistant starch is defined as the fraction of dietary starch indigested in intestines of individuals. It is considered as a functional fiber and probiotic playing significant roles in digestion physiology (Charalampopoulos et al. 2002; Sajilata et al. 2006) and positively correlated with amylose content (Raigond et al. 2015). Since molecular weight distributions and side-chain distributions of sorghum grains vary with the genetic structure (Fredriksson et al. 1998; Jane et al. 1999), sorghum genotypes should be screened through for this attribute.

Phytic acid contents of cereal grains may reduce amino acid contents and protein digestibility through complexes they formed. The ability of phytic acid to complex with proteins and inhibit enzyme activity has been reported by O' dell and De Boland (1976). It appeared that phytic acid reduced the protein digestibility by interfering with protease enzymes in groundnut (Singh et al. 1991). Reedy et al. (1982) reported that the phytate protein complex affects the protein digestibility of some cereals and legumes. Besides, they may also reduce bioavailability of important mineral cations like Fe, Zn and Ca (Bohlke et al. 2005; Selle et al. 2006). Genotype and environment have great impacts on phytic acid content of cereal grains (Raboy and Dickinson 1984).

Tannin is a phenolic compound that is found in different tissues of plants (leaves, stems, grains, etc.) and tends to form compounds with other molecules. There are two major types, hydrolyzed and condensed tannins (Butler 1989). Condensed tannins, it is formed by the condensation of flavan-3-ol. Condensed tannin is used to improve the digestive system of babies in the womb as well as giving people a feeling of satiety (Tipton et al. 1970; Ellis, 1972; Rooney and Sullins 1977; Hahn et al. 1984; Waniska et al. 1989). It has been reported that low amounts of condensed tannins (2–3%) prevent excessive and rapid breakdown of proteins in the rumen, increase fleece and milk yield of sheep, increase reproduction and have a beneficial effect on losses caused by parasites (Kamalak et al, 2005). High amounts of condensed tannins have been reported to have a detrimental effect, as they reduce the digestion of proteins, starch and fibers (Waldo 1973; Barry and Duncan 1984; Kumar and Singh 1984).

Bi-plot was first proposed by Gabriel (1971) as a graphical analysis method to present the results of principal

component analysis (PCA). This chemometric method is a scatter plot graphically displaying a rank-2 matrix by both rows (entries) and columns (testers) (Yan and Kang 2002). Using the matrix, PCA-based genotype and feature-oriented Genotype-trait bi-plot analysis was performed to visually evaluate the data matrix, interpret the relationships between traits and group genotypes. The second module of the GGE-bi-plot program was used in the bi-plot analysis (Yan 2014). In this module, the figures obtained using the paired data based on the SVD values of the standardized data obtained using the standard deviations and standard errors of the numerical values of the genotype and traits were carried on the same plot (bi-plot). Information on the model used is given in the upper right corner of the bi-plot graphics. Visual evaluation was made according to the location of the features and genotypes on the bi-plot (Akçura 2011).

New sorghum lines selected through morphological and molecular inbreeding studies from the Turkish local sorghum genotypes were used in this study. Biochemical characteristics of sorghum grains were determined and GT bi-plot analysis was used for purposeful assessment of biochemical analysis results.

Materials and methods

Field experiments and samples

In this study, 156 new sorghum lines selected from Turkish local sorghum genotypes based on morphological characteristics (plant height, stem diameter, internode distance, cluster panicle length, mid-vein color, plant color, glume color, grain color, twin seed rate, thousand-grain weight, flowering duration, ripening duration, flag leaf area, endosperm color, stay green, glume separation from the grain, glume coverage of the grain and awnness) and SSR primers and 4 registered cultivars were used.

Experiments were set up on 25 April 2015 and 1 May 2016 over the experimental fields of Erciyes University Agricultural Faculty in augmented experimental design. Experimental lines were sown in 10 replications (4 standard cultivars were replicated in each block). Each plot had 70 × 15 cm wide, 5 m long 4 rows. Fertilization was performed based on soil analysis as to have 120 kg ha⁻¹ P₂O₅ and 200 kg ha⁻¹ N. All of the phosphorus and half of nitrogen were supplied at sowing and the remaining half of nitrogen was applied when the plants reached to a height of 30–40 cm. Sprinkler irrigation was applied until the plants reached to 30–40 cm, then drip irrigations were initiated. Earthing and hoeing were performed at the same growth stage. Three hoeing and a chemical application were performed. Irrigations were performed weekly based on soil field capacity. Paper bags were used before flowering to

provide self-breeding. Seeds were harvested separately at hard-dough stage.

Soil and climate characteristics of the experimental site

Experimental years had similar temperatures with long-term averages. Precipitations were greater than the long-term averages in the first year and slightly lower in the second year. Relative humidity values of the experimental years were generally lower than the long-term averages. Unexpected low temperatures were observed in experimental years for short periods (Table 1).

Soil samples were taken from 0–30 to 30–60 cm soil profiles. Experimental soils were sand–clay in texture, slightly alkaline, poor in lime and organic matter and rich potassium and phosphorus (Table 2).

Biochemical assays

Samples harvested at hard-dough stage were ground in a mill (IKA MF 10.1, Staufen, Germany) and preserved at +4 °C

for biochemical analyses. All analyses were performed in 3 replications. Analysis results were calculated on dry matter.

Crude protein

Nitrogen content of samples (0.2 g) was determined with the Kjeldahl method. Then, the resultant nitrogen values were multiplied by 6.25 to get crude protein ratios (AOAC 1990).

Ether extract (crude oil)

Seed samples (3 g) were dissolved with ether in a soxhlet cartridge. Oil-extracted samples were then kept in a drying chamber at 95 °C for an hour, cooled in a desiccator and ether extract values were calculated with the aid of an equation (AOAC 1990).

Pepsin protein digestibility

Sample in vitro pepsin digestibility was determined in accordance with the methods specified by Aboubacar et al. (2001) and Nunes et al. (2004). Samples (200 mg) were placed into Erlenmeyer flasks and supplemented with 35 ml

Table 1 Climate data for experimental years (2015 and 2016) and long-term averages (1970–2016)

Mounts	Temperature (°C)			Precipitation (mm)			Relative humidity (%)		
	2015	2016	LT	2015	2016	LT	2015	2016	LT
April	9.4	14.1	10.8	60.4	10.3	53.2	57.1	44	61.9
May	18.2	14.7	15.1	57.2	129.2	53.2	64.2	63.5	60.6
June	22.8	20.8	19.2	98.8	30.3	40.3	44.3	51.8	55.3
July	24.3	23.5	22.6	0.5	10.4	9.9	44.9	41.7	49
August	22.1	25.3	22.1	9.6	0	6	39.4	40.2	49.3
September	13.2	17.1	17.3	0.5	21	14.5	65.5	49.5	54
October	6	12.2	11.6	23.1	4.2	30.3	61.7	51.4	63.9
Means	16.6	18.2	17				53.9	48.9	56.3
Total				250.1	205.4	207.4			

LT long-term averages

Table 2 Soil physical and chemical characteristics

Properties	2015		2016	
	0–30 cm	30–60 cm	0–30 cm	30–60 cm
Clay (%)	12.58	9.18	12.75	9.05
Silt (%)	5.11	9.55	5.27	10.65
Sand (%)	82.31	81.27	81.98	80.30
Class	Sandy-Loamy	Sandy-Loamy	Sandy-Loamy	Sandy-Loamy
pH	7.48	7.6	7.93	7.87
Organic matter (%)	1.09	1.14	1.25	1.05
CaCO ₃ (%)	0.24	0.29	0.35	0.27
K ₂ O (kg ha ⁻¹)	1184.2	842.34	1184.18	794.49
P ₂ O ₅ (kg ha ⁻¹)	110.41	12.58	98.45	12.36
EC (mmhos/cm ⁻¹)	0.83	0.27	0.72	0.23

porsin pepsin (Sigma P-7000, 890 U/mg protein activity, Sigma Chemical Co., St. Louis, MO) solution (1.5 g/L pepsin-containing 0.1 M KH_2PO_4 buffer, pH 2.0). Samples were incubated in a shaking water bath at 37 °C for 2 h and digestion was terminated with the supplementation of 2 ml 2 N NaOH. Samples were then centrifuged at 4900 g and +4 °C for 20 min and supernatant was removed. Resultant pellet was washed twice through 20 ml buffer (0.1 M KH_2PO_4 , pH 7.0) and centrifuged again. Digested nitrogen (N) was calculated with a nitrogen analysis device. Digestibility was calculated as

% digestibility

$$= (N \text{ in sample} - \text{undigested } N) / N \text{ in sample} \times 100.$$

Condense tannin

Ground samples in 1 mm sieve diameter mill 0.01 g of feed sample were weighed and put into a wide-mouthed bacteria tube. Adding 6 ml of tannin solution, it was boiled in a water bath for 1 h. After the boiling process, 3 ml of the sample was taken, and the spectrophotometer at 550 nm wavelength was read and calculated by the formula (Makkar et al. 1995).

Total, resistant and non-resistant starch content

Resistant starch contents were determined with the aid of Megazyme Resistant Starch Assay (K-RSTAR, Megazyme International Ireland Ltd, Co. Wicklow, Ireland) kit developed based on AOAC 2002. 02 Method and AACC 32–40 Method. Resistant starch and total starch values were separately obtained through determining glucose spectrophotometrically.

Amylose and amylopectin content

Megazyme Amylose and Amylopectin Analysis kit was used to determine amylose and amylopectin fractions of the starch. Starch samples were totally dispersed with dimethyl sulfoxide supplementation in a boiling water bath and precipitated with ethanol supplementation. Oils were removed through recycle of precipitate. Starch precipitate was dissolved in acetate, supplemented with conA and centrifuged to remove amylopectin. Then, amylose and total starch were hydrolyzed into D-glucose and amylose values were determined spectrophotometrically with the supplementation of glucose oxidase peroxidase. Amylopectin was determined based on the content of starch and amylose.

Phytic acid

Sample phytic acid contents were determined with the aid of phytic acid kit (K-PHYT) without a need for purification phase though ion exchange. IP6 was determined from the total phosphorus and myo-inositol phosphate forms released through phytase and phosphatase treatments and results were expressed in g/100 g.

Data analysis

Experimental data were subjected to variance analysis with SAS Software 9.0 (SAS Institute, 1999). Significant means were compared through LSD multiple comparison procedure at 1% significance level. Genotype-trait bi-plot graphs were generated to identify which genotype or genotypes were prominent with which nutrient or nutrients and to identify the sorghum genotypes to be used in further breeding studies to improve nutrient content and quality of sorghum (Yan 2014).

Results

Biochemical analysis results for sorghum lines in 2015 are provided in Table 3. The differences in biochemical characteristics of the sorghum lines were found to be highly significant ($P \leq 0.01$). Crude oil contents varied between 1.70 and 5.90%, crude protein contents between 5.73 and 14.42%, pepsin protein digestibility between 12.32 and 85.51%, condense tannin between 0.0 and 5.36%, phytic acid contents between 0.02 and 6.15%, resistant starch contents between 0.08 and 25.45%, non-resistant starch contents between 18.69 and 83.57%, total starch contents between 22.12 and

Table 3 Minimum, maximum and mean values for nutritional composition of sorghum lines in 2015*

Parameters (%)	Min	Mean	Max	Sig. Dg	LSD
Ether extract	1.70	4.17	5.90	**	0.57
Crude protein	5.73	9.39	14.42	**	1.22
Pepsin protein digestibility	12.32	57.87	85.51	**	0.38
Phytic acid	0.02	1.15	6.15	**	0.04
Condense tannin	0.0	2.16	5.36	**	0.3
Resistant starch	0.08	2.84	25.45	**	0.34
Non-resistant starch	18.69	60.76	83.57	**	1.32
Total starch	22.12	63.20	83.67	**	1.48
Amylose	4.21	14.73	32.80	**	1.61
Amylopectin	15.22	48.91	72.95	**	0.88

*Details of genotypes is presented in Supplementary Data 1, 2, 3; Sig. Dg. significant degree

** $P \leq 0.01$; LSD Least Significant Difference

83.67%, amylose contents between 4.21 and 32.80% and amylopectin contents varied between 15.22 and 72.95% (Table 3).

Biochemical analysis results for sorghum lines in 2016 are provided in Table 4. The differences in biochemical characteristics of the sorghum lines were found to be significant at 1% level. Crude oil contents varied between 1.65 and 5.65%, crude protein contents between 7.35 and 15.17%, pepsin protein digestibility between 10.49 and 77.04%, condense tannin contents between 0.18 and 5.40%, phytic acid contents between 0.02 and 6.82%, resistant starch contents between 0.11 and 23.16%, non-resistant starch contents between 11.45 and 75.85%, total starch contents between 16.46 and 79.01%, amylose contents between 3.94 and 32.37% and amylopectin contents varied between 12.50 and 65.75% (Table 4).

Table 4 Minimum, maximum and mean values for nutritional composition of sorghum lines in 2016*

Parameters (%)	Min	Mean	Max	Sig. Dg	LSD
Ether extract	1.65	3.57	5.65	**	0.56
Crude protein	7.35	10.60	15.17	**	0.98
Pepsin protein digestion	10.49	58.45	77.04	**	0.58
Condense tannin	0.18	1.91	5.40	**	0.3
Phytic acid	0.02	1.31	6.82	**	0.61
Resistant starch	0.11	2.82	23.16	**	0.39
Non-resistant starch	11.45	57.40	75.85	**	0.88
Total starch	16.46	60.22	79.01	**	1.09
Amylose	3.94	14.72	32.37	**	1.72
Amylopectin	12.50	45.47	65.75	**	0.97

*Details of genotypes is presented in Supplementary Data 1, 2, 3; Sig. Dg. significant degree

** $P \leq 0.01$; LSD Least Significant Difference

Two-year averages of biochemical analysis results for sorghum lines are provided in Table 5. Effects of the genotypes and the years on biochemical characteristics were found to be highly significant ($P \leq 0.01$). According to two-year averages, crude oil content was 3.87%, crude protein content was 9.99%, pepsin protein digestibility was 58.16%, condense tannin content was 2.04%, phytic acid content was 1.23%, resistant starch content was 2.83%, non-resistant starch content was 59.08%, total starch content was 61.91%, amylose content was 14.73% and finally amylopectin content was 47.19% (Table 5).

GT-bi-plot graphs were generated using mutual relationships between investigated biochemical traits to obtain trait separation power from chemical composition vector images (Fig. 1). According to these graphs for sorghum genotypes, amylopectin, total starch, resistant starch, non-resistant starch, condense tannin, crude protein and pepsin protein digestion were identified as the significant traits. On the other hand, crude oil, phytic acid and amylose contents had low separation power for sorghum genotypes. Bi-plot vector images reveal valuable information about the relationships among the investigated traits. There were positive correlations among amylopectin, total starch and non-resistant starch and among resistant starch and condense tannin and among amylose, crude protein, pepsin protein digestion and crude oil traits. For experimental years and average of the years, there was a negative correlation between pepsin protein digestibility and resistant starch and condense tannin. For the year 2015 and average of the years, there was also a negative correlation between amylose and resistant starch and between crude oil and resistant starch (Fig. 1). In the first year of the experiments, sorghum lines of 149, 138, 86 and 119 were found to be prominent for resistant starch, line 16 condense tannin, line 112 for phytic acid, lines 133, 135 and 20 for crude protein and pepsin protein digestibility, lines 83 and 136 for total starch and non-resistant starch

Table 5 Two-year average (2015 and 2016) minimum, maximum and mean values for nutritional composition of sorghum lines*

Parameters (%)	Min	Mean	Max	Genotype	Year	LSD
Ether extract	1.68	3.87	5.60	**	**	0.05
Crude protein	7.48	9.99	14.07	**	**	0.09
Pepsin protein digestion	11.40	58.16	79.27	**	**	0.34
Condense tannin	0.09	2.04	5.38	**	**	0.21
Phytic acid	0.02	1.23	6.49	**	**	0.30
Resistant starch	0.12	2.83	24.30	**	NS	0.03
Non-resistant starch	15.07	59.08	79.23	**	**	0.79
Total starch	19.29	61.91	79.40	**	**	0.92
Amylose	4.48	14.73	32.03	**	NS	1.18
Amylopectin	13.87	47.19	67.93	**	**	0.65

LSD Least Significant Difference, NS non-significant

*Details of genotypes are presented in Supplementary Data 1, 2, 3

** $P \leq 0.01$

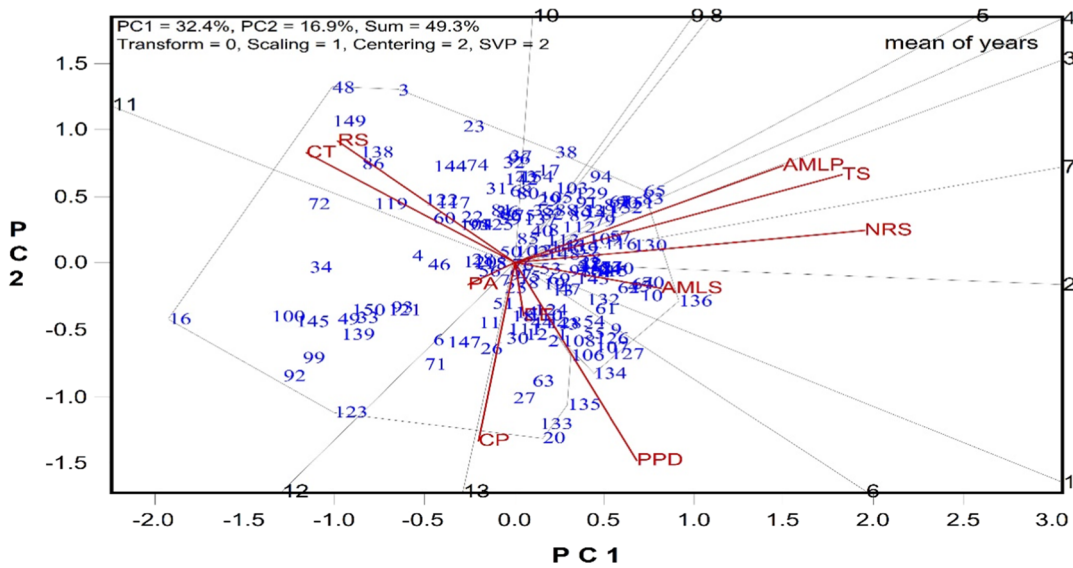
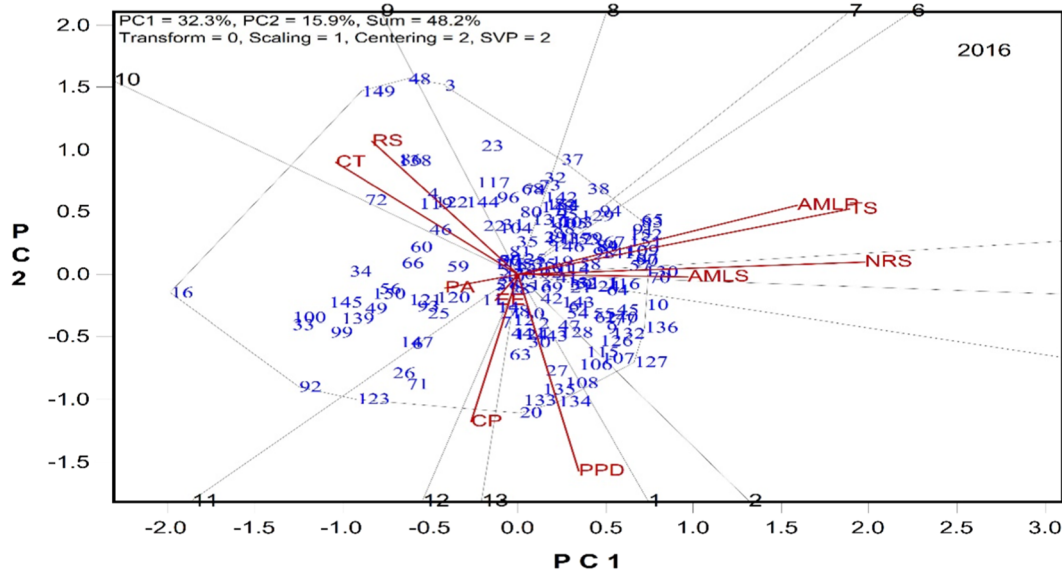
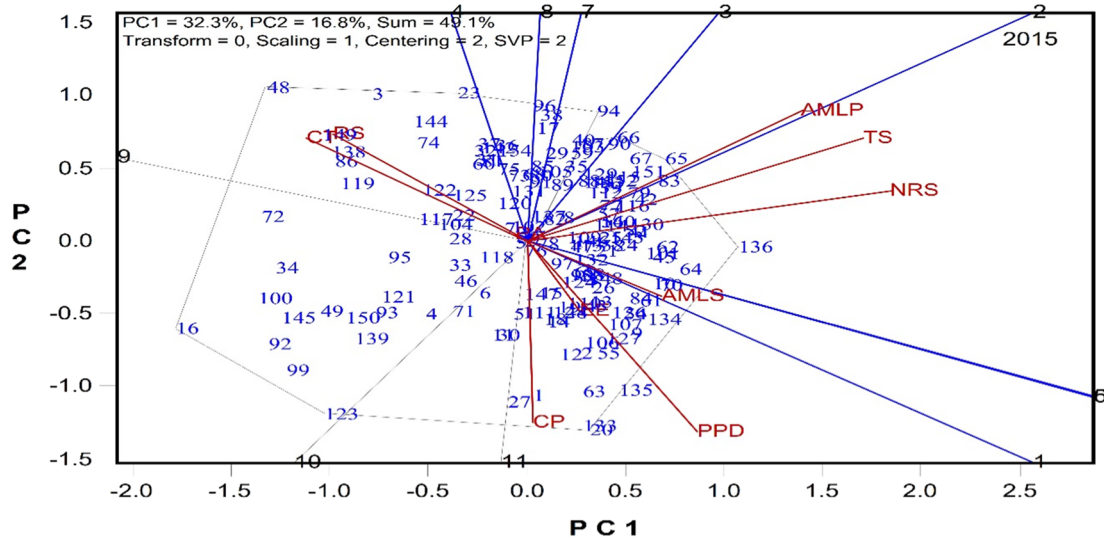


Fig. 1 Polygon views of the GT-bi-plot based on symmetrical scaling for the which-won-what pattern for genotypes and chemical composition. Details of genotypes are presented in Supplementary Data 1, 2, 3. *CP* crude protein, *EE* ether extract, *PPD* pepsin protein digestion, *PA* phytic acid, *RS* resistant starch, *NRS* non-resistant starch, *TS* total starch, *AMLS* amylose, *AMLP* amylopectin, *CT* condense tannin

and lines 65, 66 and 136 for amylopectin. In the second year of the experiments, line 57 was found to be prominent for crude oil, lines 70 and 83 for amylopectin, lines 87 and 130 for total starch and non-resistant starch and lines 94, 10 and 109 for amylopectin line 16 condense tannin. In average of the years, sorghum lines of 20, 133 and 134 were found to be prominent for crude protein and pepsin protein digestibility, line 10 for amylose, lines 65, 83 and 136 for amylopectin, total starch and non-resistant starch, line 16 condense tannin, and lines 149 and 48 for resistant starch (Fig. 1).

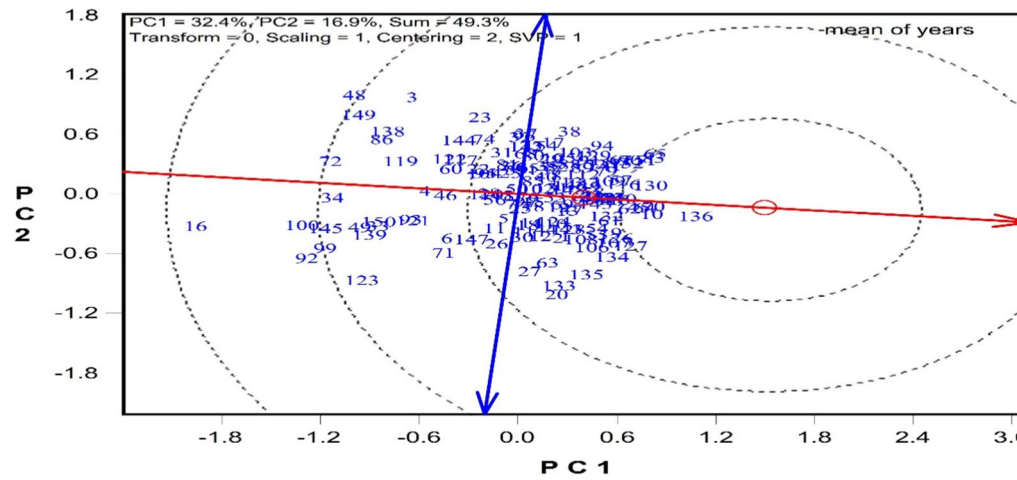
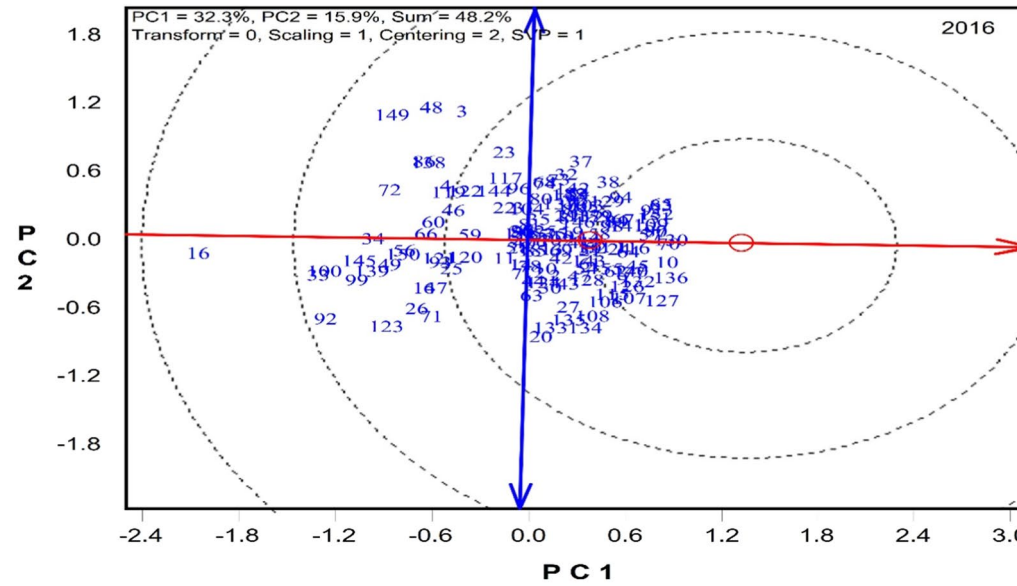
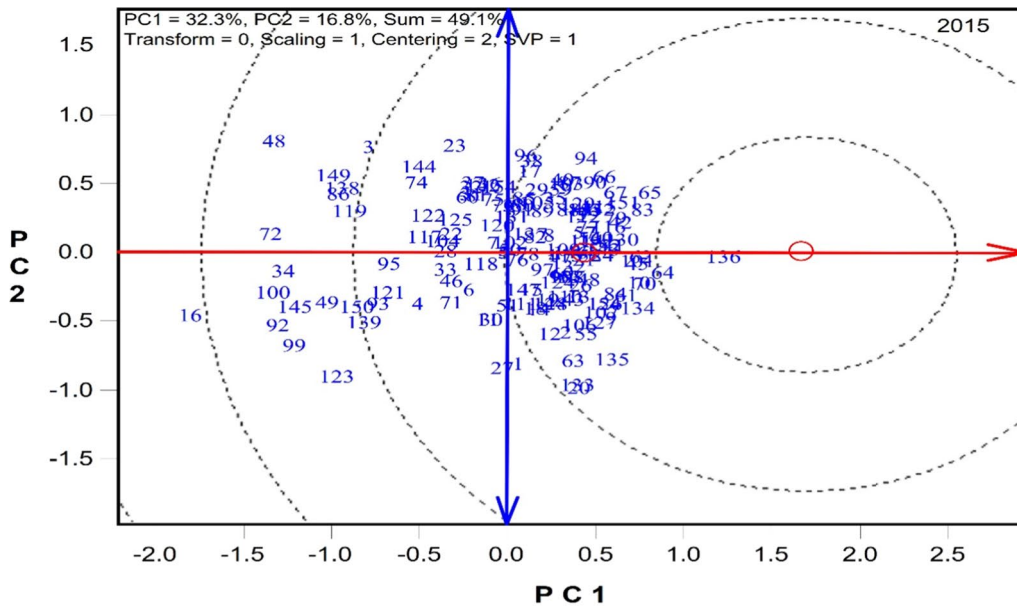
GGT-Biplot polygons allow the users to identify the ideal genotypes for investigated traits. In 2015, considering the entire traits, the lines 64 and 136 were placed at the center of the polygon and thus identified as the ideal genotypes. In 2016, the lines 127, 136, 132, 10, 70, 130, 46, 45, 94, 126 and 109 were placed at the center as ideal genotypes. With regard to average of two years, the lines 136, 10, 40, 27, 65, 130, 64, 67, 25 and 116 were identified as the ideal genotypes (Fig. 2).

Sorghum genotype biochemical profiles are presented in Fig. 3. In this bi-plot, average biochemical axis or the axis of the average biochemical coordinate (AEC) abscissa is the single-arrowed line that passes through the average biochemical and bi-plot origin. The axis of the AEC ordinate is the double-arrowed line that passes through the bi-plot origin and is perpendicular to the AEC abscissa. The sorghum lines of 48, 3, 23, 96, 94, 123, 99, 27, 1, 120, 33 and 135 in 2015, the sorghum lines of 86, 149, 48, 3, 23, 37, 92, 123, 71, 20, 31 and 34 in 2016 and the sorghum lines of 48, 3, 23, 38, 65, 94, 16, 92, 123, 20, 133, 135 and 71 in the average of years had the greatest instability for the evaluated biochemical attributes, thus they were placed on the AEC ordinate axis (Fig. 3).

Discussion

Nutritional composition of sorghum lines selected from Turkish local sorghum populations based on their morphological and molecular characteristics were compared in this study. Experiments were conducted for two years. The interactions between the investigated traits were also put forth. While the effects of years on resistant starch and amylose contents were not found to be significant, years had significant effects on the other parameters. Since there were no significant differences in average temperatures of the years, differences in these parameters were not also significant. Thusly, Kaufman et al. (2017) reported increasing amylose contents with increasing temperatures. Differences in grain-fill durations of the plants result in different starch and amylose contents (Morrison and Gadan 1987). Significant effects of cultivars and years were reported on starch, amylose, amylopectin, crude protein, crude oil, phytic acid and contents (Massaux et al. 2008; Singh et al. 2010; Wang et al. 2010). Nowotna et al. (2007) indicated that nitrogenous fertilization might have significant effects on amylose content. Warm periods, especially at ripening period, may have positive effects on starch content (Singh et al. 2010). The present research site has a dominant terrestrial climate, thus sorghum genotypes were grown under low temperatures and response of genotypes to temperature was different from each other. Therefore, they had significantly different starch contents. Present findings on total starch contents were similar with the findings of Zhang et al. (2003), Liu et al. (2012) and Rhodes et al. (2017). Amylose forms complexes with emulsifiers (monoglycerides, sucrose esters and etc.), thus is used as anti-staling and dough-improving agents. Amylose content of sorghum grains may vary greatly based on the genotype and environment (Beta et al. 2001). Amylopectin content also varies with the botanical origin, climate and soil conditions and growth stages of the grains (Singh et al. 2006). Udachan et al. (2012) reported amylose contents of sorghum grains as between 12.96 and 18.72%, Beta et al. (2001) as between 21 and 34%, Udachan et al. (2012) as between 81.28 and 89.20% and Salinas et al. (2006) as between 25.28 and 28.26%. Present findings comply with those earlier ones.

Since majority of total phosphorus in plant tissues are stored in the form of phytate, phytic acid contents are directly related to soil phosphorus (P) contents (Ravindran



◀**Fig. 2** GT-bi-plot based on genotype-focused scaling for comparison the genotypes with the ideal genotype. Details of genotypes are presented in Supplementary Data 1, 2, 3

et al. 1994; Nikolopoulou et al. 2007). Plant phosphorus uptake from the soils varies with the root morphology and genotypes (Schenk and Barber 1980), thus different plants generally have different phytic acid contents. Present phytic acid contents varied between 0.02 and 6.49%. Different from the present findings, phytic acid contents between 0.27 and 1.00% were reported in previous studies (García-Esteva et al. 1999; Elkhailil et al. 2001).

Oil content of sorghum grains with various nutritional and clinical benefits (Kaplan et al. 2018) varies greatly based on genotypes, ecological characteristics and cultural practices (Baydar 2000). Mehmood et al. (2008) reported oil contents of sorghum seeds as between 5.0 and 8.2% and Hadbaoui et al. (2010) reported oil contents of sorghum grains as between 11 and 13%. Present values varied between 1.68 and 5.60%.

Crude protein is a significant quality indicator for food-stuffs (Assefa and Ledin 2001). Singh et al. (1987) reported protein contents of sorghum grains as between 9.90 and 19.80%, FAO (1995) and Beta et al. (1995) reported crude protein contents as between 7 and 15%. Differences in dry matter yields and protein contents of the plants are generally attributed to genetic structures, ripening periods, temperature and fertilization practices (Ball et al. 2001). Chemical composition and nutritional values of sorghum grains are influenced by the genotype, climate, soil texture and fertilization (Ebadi et al. 2005). Kafirins constitute about 50–70% of sorghum proteins and they reduce digestion (Duodu et al. 2003). Disulphur cross-links of kafirin are greater than corn proteins and such a case reduce digestible protein ratios (Salinas et al. 2006; Selle et al. 2010). The differences in alpha, beta and gamma kafirin quantities of sorghum grains result in differences in protein digestibility of sorghum genotypes (Hicks et al. 2001). Since reduced protein digestibility generates serious nutrient deficiencies in humans directly fed with sorghum, generally the genotypes with greater digestible protein ratios are tried to be selected in sorghum selection studies.

Condensed tannins of sorghum are antioxidants, which slow hydrolysis in foods, produce naturally dark-colored products and increase the dietary fiber levels of food products and tannin levels vary among genotypes (Dykes and Rooney 2006). Waghorn, (1990), the first negative effect seen in animals fed with feeds containing more than 60 g of tannins per kilogram is disgusting and other harmful effects are seen above this amount. None of the sorghum genotypes used in our study has such high tannin content and can be used easily in feed rations. Condensed tannin contents obtained in this study are similar to Salinas et al. (2006), Gurbuz and Davies (2010), Kaplan and Kızıllımshek (2012) and Hermuth and Kosová (2017).

Bi-plot analysis can be applied to all genotypes equally through inputting genotype trait like two-way data (Akcura et al. 2011). With bi-plot analysis, genotypes can be screened through for different traits (Yan and Tinker 2006) and ideal genotypes can be identified for investigated traits (Yan and Kang 2003). The ideal genotype is the one located in central circle of the one closest to central circle (Kaya et al. 2006). In present study, same procedures were employed for the selection of ideal genotypes. In 2015, considering the entire traits, the lines 64 and 136 were placed at the center of the polygon and thus identified as the ideal genotypes. In 2016, the lines 127, 136, 132, 10, 70, 130, 46, 45, 94, 126 and 109 were placed at the center as ideal genotypes. With regard to average of two years, the lines 136, 10, 40, 27, 65, 130, 64, 67, 25 and 116 were identified as the ideal genotypes (Fig. 2). Bi-plot analysis also allows the users to visually assess the relationships between the traits. Since the cosine of the angle between the vectors of any two traits approximates the correlation coefficient between them, this view of the bi-plot is the best for visualizing the interrelationship among the traits (Yan and Kang 2003). Similar with the present findings, Boyles et al. (2017) reported positive correlations of starch with crude protein and crude oil. The information about the relationships between starch and protein digestibility is quite conflicting because of the differences in starch gelling. Duodu et al. (2003) reported a negative correlation between resistant starch and protein digestibility. A negative correlation was also observed between these two parameters of the present study (Fig. 1).

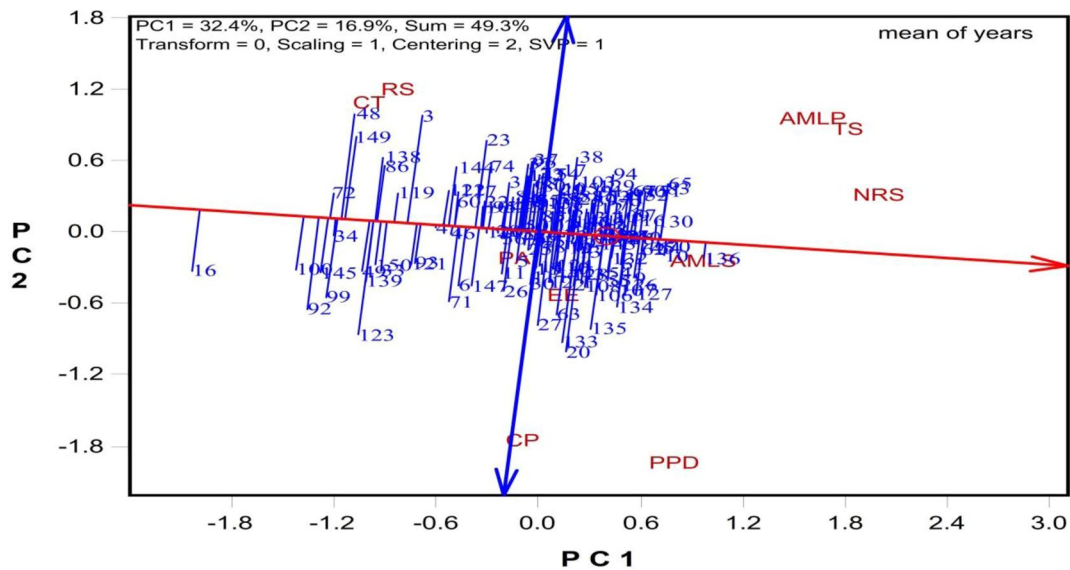
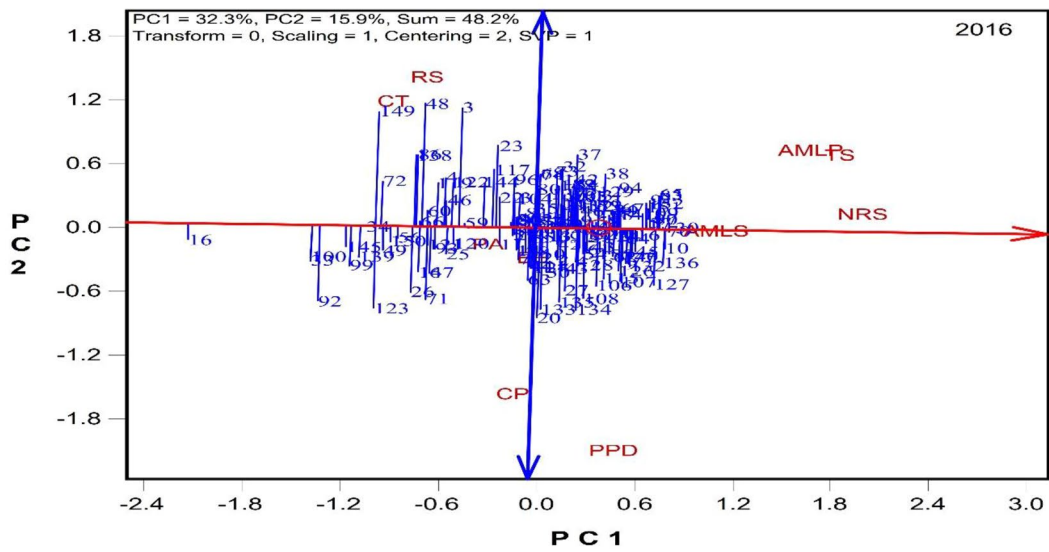
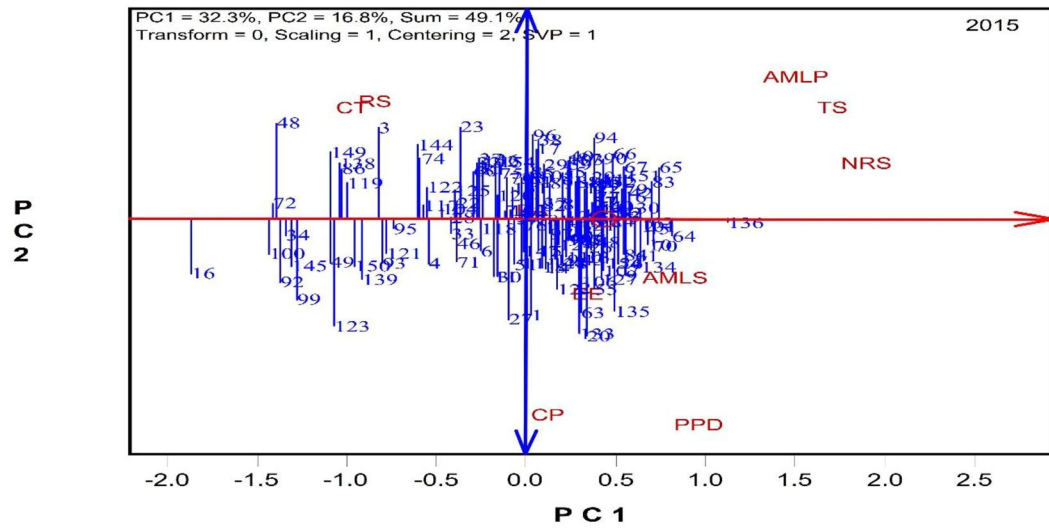


Fig. 3 Genotype ranking based on biochemical properties. Details of genotypes are presented in Supplementary Data 1, 2, 3. *CP* crude protein, *EE* ether extract, *PPD* pepsin protein digestion, *PA* phytic acid, *RS* resistant starch, *NRS* non-resistant starch, *TS* total starch, *AMLS* amylose, *AMLPL* amylopectin, *CT* condense tannin

Conclusion

Present findings revealed a great variation in protein, oil, condense tannin, starch and phytic acid-like biochemical attributes of sorghum genotypes. Majority of selected sorghum lines had superior protein, oil, condense tannin, total starch, amylopectin and resistant starch values than the standard cultivars. It was concluded based on present findings that identification of different attributes of local genotypes and lines was a significant issue from the point of human needs. GT bi-plot analysis quite facilitated the interpretation of the results. With GT bi-plot, visual data presentation was provided and stability of sorghum lines was determined easily. In brief, GT bi-plot facilitated the selection processes.

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References

- Aboubacar A, Axtell JD, Huang CP, Hamaker BR (2001) A rapid protein digestibility assay for identifying highly digestible sorghum lines. *Cereal Chem* 78(2):160–165
- Afify AEMMR, El-Beltagi HS, Abd El-Salam SM, Omran AA (2011) Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white sorghum varieties. *PLoS ONE* 6:255–212
- Akçura M (2011) The relationships of some traits in Turkish winter bread wheat landraces. *Turk J Agric* for 35(2):115–125
- Akcura M, Taner S, Kaya Y (2011) Evaluation of bread wheat genotypes under irrigated multi-environment conditions using GGE biplot analyses. *Zemdirbyste-Agric* 98(1):35–40
- Akintayo I, Sedjo J (eds) (2001) Towards sustainable sorghum production and utilization in West and Central Africa. WASRN/ICRISAT, Lomé Togo, p 162 (19-22 April 1999 ISBN 92-9066-4330-9)
- AOAC (1990) Official methods of analysis, 15th edn. Association of Official Analytical Chemists, Arlington, VA
- Assefa G, Ledin I (2001) Effect of variety, soil type and fertiliser on the establishment, growth, forage yield, quality and voluntary intake by cattle of oats and vetches cultivated in pure stands and mixtures. *Anim Feed Sci Technol* 92(1–2):95–111
- Ball DM, Collins M, Lacefield GD, Martin NP, Mertens DA, Olson KE, Putnam DH, Undersander DJ, Wolf MW (2001) Understanding forage quality. American Farm Bureau Federation Publication, 1-01, Park Ridge, IL
- Barry TN, Duncan SJ (1984) The role of condensed tannin in the nutritional value of lotus pedunculatus for sheep 1. Voluntary intake. *Br J Nutr* 51:485–491
- Baydar H (2000) Effects of gibberellic acid on male sterility, seed yield and oil and fatty acid syntheses of safflower (*Carthamus tinctorius* L.). *Turk j Biol* 24(1):159–168
- Beta T, Rooney LW, Waniska RD (1995) Malting characteristics of sorghum cultivars. *Cereal Chem* 72(6):533–538
- Beta T, Corke H, Rooney LW, Taylor JRN (2001) Starch properties as affected by sorghum grain chemistry. *J Sci Food Agric* 81(2):245–251
- Bohlke RA, Thaler RC, Stein HH (2005) Calcium, phosphorus, and amino acid digestibility in low-phytate corn, normal corn, and soybean meal by growing pigs. *J Anim Sci* 83:2396–2403
- Boyles RE, Pfeiffer BK, Cooper EA, Rauh BL, Zielinski KJ, Myers MT, Brenton Z, Rooney WL, Kresovich S (2017) Genetic dissection of sorghum grain quality traits using diverse and segregating populations. *Theor Appl Genet* 130(4):697–716
- Bray GA, Popkin BM (2014) Dietary sugar and body weight: have we reached a crisis in the epidemic of obesity and diabetes? Health be damned! Pour on the sugar. *Diabetes Care* 37(4):950–956
- Butler LG (1989) Effects of condensed tannin on animal nutrition. Chemistry and significance of condensed tannins. Springer, Boston, MA, pp 391–402
- Charalampopoulos D, Wang R, Pandiella SS, Webb C (2002) Application of cereals and cereal components in functional foods: a review. *Int J Food Microbiol* 79:131–141. [https://doi.org/10.1016/S0168-1605\(02\)00187-3](https://doi.org/10.1016/S0168-1605(02)00187-3)
- FAO (1995) Millets in Human nutrition. FAO Food Nutri Serie 27:16–19
- de Moraes CL, Pinheiro SS, Martino HSD, Pinheiro-Sant'Ana HM (2017) Sorghum (*Sorghum bicolor* L.), Nutrients, bioactive compounds, and potential impact on human health. *Crit Rev Food Sci Nutr* 57(2):372–390
- Duodu KG, Taylor JRN, Belton PS, Hamaker BR (2003) Factors affecting sorghum protein digestibility. *J Cereal Sci* 38(2):117–131
- Dykes L, Rooney LW (2006) Sorghum and millet phenols and antioxidants. *J Cereal Sci* 44(3):236–251
- Ebadi MR, Pourreza J, Jamalian J, Edriss MA, Samie AH, Mirhadi SA (2005) Amino acid content and availability in low, medium and high tannin sorghum grain for poultry. *Int J Poult Sci* 4(1):27–31
- Elkhalil EAI, El Tinay AH, Mohamed BE, Elsheikh EAE (2001) Effect of malt pretreatment on phytic acid and in vitro protein digestibility of sorghum flour. *Food Chem* 72(1):29–32
- Ellis EB (1972) Morphological characteristics in relation to seed deterioration in sorghum. M.S. Thesis Texas A&M University College Station TX.
- Ezeogu LI, Duodu KG, Taylor JRN (2005) Effects of endosperm texture and cooking conditions on the in vitro starch digestibility of sorghum and maize flours. *J Cereal Sci* 42(1):33–44. <https://doi.org/10.1016/j.jcs.2005.02.002>
- Fredriksson H, Silverio J, Andersson R, Eliasson AC, Aman P (1998) The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches. *Carbohydr. Polym* 35:119–134
- Gabriel KR (1971) The biplot graphic display of matrices with application to principal component analysis. *Biometrika* 58(3):453–467

- García-Esteva RM, Guerra-Hernández E, García-Villanova B (1999) Phytic acid content in milled cereal products and breads. *Food Res Int* 32(3):217–221
- Gurbuz Y, Davies DR (2010) Organic matter digestibility and condensed tannin content of hybrid sorghum. *Animal Nutr Feed Technol* 10(1):19–28
- Hadbaoui Z, Djeridane A, Yousif M, Saidi M, Nadjemi B (2010) Fatty acid, tocopherol composition and the antioxidant activity of the lipid extract from the sorghum grains growing in Algeria 3:215–220
- Hahn DH, Rooney LW, Earp CF (1984) Tannins and phenols of sorghum. *Cereal Foods World* 29:776–779
- Hermuth J, Kosová K (2017) Characterization of the first Czech sorghum variety ruzrok tested in the Czech Republic. *Czech J Genet Plant Breed* 53(1):37–44
- Hicks C, Bean SR, Lookhart GL, Pedersen JF, Kofoid KD, Tuinstra MR (2001) Genetic analysis of kafirins and their phenotypic correlations with feed quality traits, *in vitro* digestibility, and seed weight in grain sorghum. *Cereal Chem* 78(4):412–416
- Jane J, Chen YY, Lee LF, McPherson AE, Wong KS, Radosavljevic M, Kasemsuwan T (1999) Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chem* 76:629–637
- Kamalak A, Canbolat Ö, Gürbüz Y, Özyay O, Erer M, Özkan ÇÖ (2005) Kondense Taninin Rumimant Hayvanlar Üzerindeki Etkileri Hakkında bir İnceleme. *KSÜ Fen Ve Mühendislik Dergisi* 8(1):132–137
- Kaplan M, Kızılsimşek M (2012) Farklı tane sorgum (*Sorghum bicolor* L.) hat ve çeşitlerinin besleme değerlerinin belirlenmesi. *Erciyes Üniv Fen Bilimleri Enstitüsü Dergisi* 28(1):11–14
- Kaplan M, Temizgül R, Büyükkılıç Beyzi S, Kokten K, Karaman K (2018) Classification of different *Sorghum bicolor* genotypes depending on fatty acid composition with using biplot analysis. *Prog Nutr* 4:1–7
- Kaufman RC, Wilson JD, Bean SR, Xu F, Shi YC (2017) Sorghum starch properties as affected by growing season, hybrid, and kernel maturity. *J Cereal Sci* 74:127–135
- Kaya Y, Akcura M, Taner S (2006) GGE-biplot analysis of multi-environment yield trials in bread wheat. *Turk J Agric for* 30:325–337
- Kumar R, Singh M (1984) Tannins: their adverse role in ruminant nutrition. *Agric Food Chem* 32:447–453
- Liu L, Herald TJ, Wang D, Wilson JD, Bean SR, Aramouni FM (2012) Characterization of sorghum grain and evaluation of sorghum flour in a Chinese egg noodle system. *J Cereal Sci* 55(1):31–36
- Makkar HPS, Blümmel M, Becker K (1995) Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in *in vitro* techniques. *Br J Nutr* 73(6):897–913
- Massaux C, Sindic M, Lenartz J, Sinnaeve G, Bodson B, Falisse A, Dardenne P, Deroanne C (2008) Variations in physicochemical and functional properties of starches extracted from European soft wheat (*Triticum aestivum* L.): the importance to preserve the varietal identity. *Carbohydr Polym* 71:32–41
- Mehmood S, Orhan I, Ahsan Z, Aslan S, Gulfraz M (2008) Fatty acid composition of seed oil of different *Sorghum bicolor* varieties. *Food Chem* 109:855–859
- Morrison WR, Gadan H (1987) The amylose and lipid contents of starch granules in developing wheat endosperm. *J Cereal Sci* 5:263–275
- Nikolopoulou D, Grigorakis K, Stasini M, Alexis MN, Iliadis K (2007) Differences in chemical composition of field pea (*Pisum sativum*) cultivars: Effects of cultivation area and year. *Food Chem* 103(3):847–852. <https://doi.org/10.1016/j.foodchem.2006.09.035>
- Nowotna A, Gambuś H, Krytech G, Krawontka J, Gambuś F, Sabat R, Ziobro R (2007) Effect of nitrogen fertilization on the physico-chemical properties of starch isolated from German triticale varieties. *Starch-Stärke* 59:397–399
- Nunes A, Correia I, Barros A, Delgadillo I (2004) Sequential *in vitro* pepsin digestion of uncooked and cooked sorghum and maize samples. *J Agric Food Chem* 52(7):2052–2058
- O'Dell BL, De Boland A (1976) Complexation of phytate with proteins and cations in corn and oilseed meals. *J Agric Food Chem* 24:804–808
- Raboy V, Dickinson DB (1984) Effect of phosphorus and zinc nutrition on soybean seed phytic acid and zinc. *Plant Physiol* 75(4):1094–1098
- Raigond P, Ezekiel R, Raigond B (2015) Resistant starch in food: a review. *J Sci Food Agric* 95(10):1968–1978
- Ravindran V, Ravindran G, Sivalogan S (1994) Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. *Food Chem* 50:133–136
- Reddy NR, Sathe SK, Salunkhe DK (1982) Phytates in legumes and cereals. *Adv Food Res* 28:1–90
- Rhodes DH, Hoffmann L, Rooney WL, Herald TJ, Bean S, Boyles R, Kresovich S (2017) Genetic architecture of kernel composition in global sorghum germplasm. *BMC Genom* 18(1):15
- Rooney LW, Sullins RD (1977) The structure of sorghum and its relation to processing and nutritional value. In: Dendy DAV (ed) Proceedings of a symposium on sorghums and millets for human food. Tropical Products Institute, London, pp 91–109
- Sajilata MG, Singhal RS, Kulkarni PR (2006) Resistant starch: a review. *Compr Rev Food Sci Food Saf* 5:1–17
- Salinas I, Pró A, Salinas Y, Sosa E, Becerril CM, Cuca M, Cervantes M, Gallegos J (2006) Compositional variation amongst sorghum hybrids: effect of kafirin concentration on metabolizable energy. *J Cereal Sci* 44(3):342–346
- Schenk MK, Barber SA (1980) Potassium and phosphorus uptake by corn genotypes grown in the field as influenced by root characteristics. *Plant Soil* 54(1):65–76
- Selle PH, Ravindran V, Bryden WL, Scott T (2006) Influence of dietary phytate and exogenous phytase on amino acid digestibility in poultry. A review. *J Poult Sci* 43:89–103
- Selle PH, Cadogan DJ, Li X, Bryden WL (2010) Implications of sorghum in broiler chicken nutrition. *Anim Feed Sci Technol* 156(3–4):57–74
- Singh P, Singh U, Eggum BO, Kumar KA, Andrews DJ (1987) Nutritional evaluation of high protein genotypes of pearl millet [*Pennisetum americanum* (L.) Leeke]. *J Sci Food Agric* 38(1):41–48
- Singh U, Singh B, Smith OD (1991) Effect of varieties and processing methods on phytic acid and protein digestibility of groundnut (*Arachis hypogaea*). *J Food Sci Tech* 28:345–347
- Singh N, Kaur L, Sandhu KS, Kaur J, Nishinari K (2006) Relationships between physicochemical, morphological, thermal, rheological properties of rice starches. *Food Hydrocoll* 20(4):532–542
- Singh S, Gupta AK, Gupta SK, Kaur N (2010) Effect of sowing time on protein quality and starch pasting characteristics in wheat (*Triticum aestivum* L.) genotypes grown under irrigated and rain-fed conditions. *Food Chem* 122(3):559–565
- Tipton KW, Floyd EH, Marshall JG, McDevitt JB (1970) Resistance of certain grain sorghum hybrids to bird damage in Louisiana. *Agron J* 62:211–213
- Udachan IS, Sahu AK, Hend FM (2012) Extraction and characterization of sorghum (*Sorghum bicolor* L. Moench) starch. *Int Food Res J* 19(1):315–319
- United States Grains Council (2001) Sorghum production and usage data, Available from: <http://www.grains.org/grains/sorghum>. Html
- Waghorn GC (1990) Bloat in cows grazing leguminous pastures. *Proc Soc Animal Prod* 18:412–415
- Waldo DR (1973) Extent and partition of cereal grain starch digestion in ruminants. *J Anim Sci* 37(4):1062–1074

- Wang N, Hatcher DW, Warkentin TD, Toews R (2010) Effect of cultivar and environment on physicochemical and cooking characteristics of field pea (*Pisum sativum*). *Food Chem* 118(1):109–115
- Waniska RD, Poe JH, Bandyopadhyay R (1989) Effects of growth conditions on grain molding and phenols in sorghum caryopsis. *J Cereal Sci* 10:217–225
- Wong JH, Lau T, Cai N, Singh J, Pedersen JF, Vensel WH (2009) Digestibility of protein and starch from sorghum (*Sorghum bicolor*) is linked to biochemical and structural features of grain endosperm. *J Cereal Sci* 49(1):73–82
- Yan W (2014) *Crop variety trials: data management and analysis*. Wiley Blackwell. <http://ca.wiley.com/WileyCDA/WileyTitle/productCd-1118688643.html>
- Yan W, Kang MS (2003) *GGE-biplot analysis: a graphical tool for breeders*. Geneticists and agronomists. CRD Press, Boca Raton
- Yan W, Tinker NA (2006) Biplot analysis of multi-environment trial data: principles and applications. *Can J Plant Sci* 86(3):623–645
- Yan and Kang (2002) *GGE biplot analysis: a graphical tool for breeders, geneticists, and agronomists* CRC Press. <http://www.crcpress.com/product/isbn/9780849313387>
- Yousif A, Nhepera D, Johnson S (2012) Influence of sorghum flour addition on flat bread in vitro starch digestibility, antioxidant capacity and consumer acceptability. *Food Chem* 134:880–887
- Zhang X, Wang D, Tuinstra MR, Bean S, Seib PA, Sun XS (2003) Ethanol and lactic acid production as affected by sorghum genotype and location. *Ind Crops Prod* 18:45–255

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