

Classification of different *Sorghum bicolor* genotypes depending on fatty acid composition with using Biplot Analysis

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Summary. Seed fatty acid composition and oil content of 61 sorghum genotypes were analyzed by gas chromatography (GC) system and genotypes were classified with Biplot analysis. The fatty acid composition of studied plants showed different saturated and unsaturated fatty acid concentrations. The total oil contents in the seeds of sorghum genotypes ranged from 2.32 to 5.74%. The major fatty acids were found to be linoleic acid (29.85-51.95%), oleic acid (30.62-49.73%), palmitic acid (10.96-22.02%), stearic acid (1.36-7.32%) and linolenic acid (0.58-5.41%), Biplot analysis was utilized to characterize and classify genotypes depending on the fatty acid composition. Two principal components (PCs) were obtained from analysis and determined to be explanatory of more than 82.76% of the total variability in the data set for fatty acids. And also significant negative and positive correlations were found among fatty acids such as between palmitic and stearic acids ($r=0.821$). The present study is the first report on oil content and fatty acid composition of local sorghum landraces from Turkey.

Keywords: Oleic acid, Linoleic acid, Linolenic acid, Oil content, *Sorghum bicolor*.

Introduction

Sorghum is grown especially in less precipitated, tropic, sub-tropic and temperate climate zones of the world. It has a great genetic diversity (with more than 40 000 species) and is commonly used as food, feed and industrial crop (Rooney 2004). Sorghum is produced in various regions of America, Africa, India and China. Sorghum grains are used to meet energy, protein, vitamin and mineral needs of millions of poor people. They are used either fermented or non-fermented fashions in breads, sorghum flakes, alcoholic beverages and beers. Sorghum shoots and leaves are used as animal feed and construction materials (Rajvanshi and Nimbkar 2001; Rooney 2000). As compared to starch and protein content, sorghum grains have low oil contents (about 3-5%). However, since it

is quite tolerant to drought and high temperatures, has high yields with low input costs, it can be used as an alternative oil source with clinical advantages (Mehmood et al. 2008).

Both genetic structure and environmental factors greatly influence oil content and quality of oil crops (Baenziger et al. 2001). Fatty acids of the seeds are highly significant for oil industry. Fatty acids can either be mono-unsaturated (MUFA) or polyunsaturated (PUFA) (Kostik et al. 2015). On the other hand, unsaturated ones are also classified as omega. Although $\omega 9$ is not essential for humans, $\omega 3$ and $\omega 6$ are essential since they can be synthesized by mammals and they should be supplied in human diets (Ristic and Risic 2003; Assiesa et al. 2004).

Vegetable oils not only supply a high-quality nutrient, but also supply essential nutrients and clini-

cally important bioactive compounds. For instance, PUFAs exist both as component of membrane phospholipids in specific tissues and as precursor of hormone-like prostaglandins (Patil and Gislerod 2006). Saturated fatty acids increase the risk of cardiovascular diseases, cancer and autoimmune diseases. When the ratio of unsaturated fatty acids was higher than the ratio of saturated fatty acids, the nutritional value of oils as a lipid source is higher (Iso et al. 2002; Aronson et al. 2001). Recently, pharmacologically significant fatty oils have attracted the attentions of both the consumers and producers (Mehmood et al. 2008). With regard to this issue, sorghum is the most remarkable plant and popularity of sorghum is ever-increasing. Biplot analysis is commonly used for visual presentation of the relationships among various attributes of the genotypes (Yan et al. 2001). It is also used to identify superior genotypes and their prominent attributes (Yan and Kang 2003). Therefore

Biplot analysis in quite facilitate assessment of the research findings (Yan 2014).

The present study was conducted to characterize different local sorghum (*Sorghum bicolor* L.) with regard to crude oil and fatty acid compositions and to assess the applicability of Biplot for the classification of landraces depending on their fatty acid composition.

Materials and Methods

Seed samples

In this study, a total of 58 sorghum (*Sorghum bicolor*) genotypes from Plant Germplasm System (USDA) and International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) and Eagan Agricultural Research Institute (ETAE) and 3 standard varieties (Rox, Beydari and SC 405) were used as the plant material (Table 1). The seeds supplied

Table 1. Codes and abbreviations of sorghum landrace

Landrace No	Gen Bank Code	Landrace No	Gen Bank Code	Landrace No	Gen Bank Code
SL1	IS 12786	SL22	IS 12852	SL43	IS 13205
SL2	IS 12845	SL23	IS 12817	SL44	TR 38374
SL3	IS 12833	SL24	Burdur	SL45	TR 38372
SL4	IS 12821	SL25	Urfa	SL46	TR 38351
SL5	IS 12849	SL26	IS 41745	SL47	TR 38252
SL6	IS 12796	SL27	IS 3081	SL48	TR 38278
SL7	IS 12848	SL28	IS 41736	SL49	TR 38282
SL8	IS 12801	SL29	IS 2373	SL50	TR 38429
SL9	IS 13155	SL30	IS 3085	SL51	TR 38244
SL10	IS 12816	SL31	IS 41744	SL52	Kilis
SL11	IS 12837	SL32	IS 41738	SL53	Hatay
SL12	IS 12862	SL33	IS 2374	SL54	IS 12827
SL13	IS 12785	SL34	IS 2883	SL55	PI 166979 03
SL14	IS 20865	SL35	IS 2889	SL56	PI 173114 01
SL15	IS 12822	SL36	IS 2885	SL57	PI 344084 02
SL16	IS 12818	SL37	IS 12856	SL58	Antep
SL17	IS 12819	SL38	IS 21863	Std	Rox
SL18	IS 12850	SL39	IS 12894	Std	Beydari
SL19	IS 12855	SL40	IS 13150	Std	SC 405
SL20	IS 12858	SL41	IS 13203		
SL21	IS 12808	SL42	IS 13211		

Std: standart genotypes

from gene banks were sown and propagated under control conditions of Kayseri province of Turkey (39°48'N; 38°73'E). Resultant plants were subjected to relevant analyses.

Oil extraction and preparation of fatty acid methyl esters (FAME)

Impurities were removed from the seeds and the cleaned seeds were ground using a ball mill into powder. Lipids were extracted with hexane/isopropanol (2 v/v) solution at overnight in laboratory type shaker. The lipid extracts were centrifuged at 10000 g for 5 min and filtered after that solvent was removed on a rotary evaporator at 40 °C. After extraction procedure, fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulphuric acid (v/v) in methanol (Christie 1990).

Determination of fatty acid composition by GC system

Lipid fatty acid methyl esters (FAME) in feed samples were prepared using 1-step extraction-trans esterification. The FAME profile for a 0.6- μ L sample at a split ratio of 1:50 was generated using a gas chromatograph (Schimadzu, GC 2010 plus) equipped with a flame ionization detector (Schimadzu, Kyoto, Japan), a 100-m fused silica capillary column (i.d. 0.25 mm) and H₂ as the carrier and fuel gas. The FAME were separated using a temperature gradient program (Chilliard et al. 2013), and the peaks were identified based on comparing retention times with authentic standard (Supelco #37, Supelco Inc., Bellefonte, PA, USA; L8404 and O5632; Sigma).

Statistical analysis

Data were subjected to variance analysis with SAS (SAS Inst. 1999) statistical software. LSD multiple range test was employed to compare the treatment means as a complement of ANOVA procedure. Biplot analysis was performed using fatty acid compositions as variables and sorghum genotypes as classification criterion. The Biplot analysis was achieved using XLSTAT Software (XLSTAT, 2008, Add in soft, New York, NY, USA).

Results and Discussion

In this study, oil content and fatty acid composition of 58 lines and 3 varieties of *Sorghum bicolor* L. were detected and the results were shown in Table 2. Oil content of studied genotypes was between 2.32% and 5.74% (Table 2). SL50 genotype (5.74%), SL19 genotype (5.45%) and SL21 genotype (5.22%) have the highest oil content. The lowest percentage was found in SC405 variety. Osman et al. (2000) determined that IS2284 sorghum variety has highest level of oil content (5.63%) and also Mehmood et al. (2008) reported that the highest percentage of oil among the ten varieties was 8.4% found in seed of variety 86-G-87. The oil content of sorghum obtained in our work agreed with that reported by Liu (2011).

The fatty acid composition of studied plants used as feed crops showed different concentrations of saturated and unsaturated fatty acids. The main components in the seed oils of these genotypes are linoleic (C18:2), linolenic (C18:3), oleic (C18:1), stearic (C18:0) and palmitic (C16:0) acid. The palmitic acid in seeds of 61 genotypes of sorghum ranged from 10.96 to 22.02% (Table 2). From the data presented it could be seen that the highest palmitic acid was found in SL31 genotype, while the lowest percentage was found in SL7 genotype. The mean values of palmitic acid of sorghum genotypes obtained in our work agreed with the previous reports (Osman et al. 2000; Cherian et al. 2002; Mehmood et al. 2008; Liu 2011). The palmitic acid was higher in 3 genotypes (SL31, SL37 and SL56) as compared to the other genotypes.

The results in Table 2 also indicate that the content of stearic acid ranged from 1.36 to 7.32%. Stearic acid (18:0) was the highest saturated fatty acid (SFA) in SL37 genotype (7.32%), SL24 genotype (6.61%), SL25 genotype (6.54%), SL31 genotype (5.92%) and SL56 genotype (5.70%) respectively. The lowest percentage of stearic acid was found in SL14 and SL39 genotypes (1.36%) (Table 2). The mean values of stearic acid of sorghum genotypes obtained in our work agreed with studies reported by Osman et al. (2000), Cherian et al. (2002), Mehmood et al. (2008), Liu (2011).

Oleic acid was found in high quantities in all genotypes. It ranged from 30.62 to 49.73% in all genotypes.

Table 2. Fatty acid composition and oil content of studied samples (%)

Fatty acid	SL1	SL2	SL3	SL4	SL5	SL6	SL7	SL8	SL9	SL10	SL11	SL12	SL13	SL14	SL15	SL16	SL17
Oil content	4.51	3.14	4.27	4.71	4.48	3.08	2.83	4.44	4.48	2.82	4.37	4.44	4.15	4.24	2.74	3.19	3.42
C 16:0	12.13	13.72	12.62	12.94	13.63	13.63	10.96	15.49	13.65	12.39	13.91	11.61	13.62	12.13	19.89	13.64	11.65
C 18:0	2.46	2.11	1.96	1.67	1.84	2.41	1.98	4.11	1.66	2.62	1.80	1.55	1.88	1.36	3.75	1.66	1.51
C 18:1	42.57	41.09	42.77	42.06	40.41	43.31	43.84	39.75	44.43	35.17	37.54	40.05	40.05	36.20	39.49	36.32	39.05
C 18:2	41.94	42.07	41.30	41.72	42.38	38.88	41.76	37.80	39.03	47.94	45.08	45.60	42.95	49.09	35.77	47.21	46.63
C 18:3	0.91	1.00	1.34	1.61	1.73	1.77	1.46	2.84	1.23	1.88	1.67	1.19	1.50	1.22	1.10	1.17	1.16
ΣTSEFA	14.59	15.83	14.58	14.61	15.47	16.04	12.94	19.60	15.31	15.01	15.71	13.17	15.50	13.49	23.64	15.29	13.16
ΣTUSFA	85.41	84.17	85.42	85.39	84.53	83.96	87.06	80.40	84.69	84.99	84.29	86.83	84.50	86.51	76.36	84.71	86.84
Oil content	4.41	5.45	4.28	5.22	4.03	3.64	3.22	4.69	2.77	3.38	4.26	4.32	2.82	2.65	3.06	3.32	2.72
C 16:0	16.26	13.29	13.27	12.45	15.92	13.89	17.91	17.08	12.63	13.04	13.89	13.93	13.82	22.02	16.00	12.50	15.82
C 18:0	2.62	2.31	1.73	1.80	3.83	2.55	6.61	6.54	2.10	3.04	2.24	2.24	2.86	5.92	4.23	1.77	2.56
C 18:1	40.05	39.75	41.09	38.87	43.05	42.51	35.26	41.12	39.76	43.44	44.20	44.17	49.73	36.51	33.81	38.38	43.94
C 18:2	39.71	43.65	42.61	45.25	36.49	39.53	37.47	29.85	43.29	39.53	38.07	38.08	32.93	32.47	44.49	46.44	36.98
C 18:3	1.36	1.00	1.30	1.64	0.71	1.53	2.76	5.41	2.22	0.94	1.60	1.59	0.66	3.09	1.48	0.91	0.70
ΣTSEFA	18.88	15.60	15.00	14.24	19.75	16.44	24.51	23.62	14.73	16.09	16.13	16.16	16.69	27.93	20.23	14.27	18.38
ΣTUSFA	81.12	84.40	85.00	85.76	80.25	83.56	75.49	76.38	85.27	83.91	83.87	83.84	83.31	72.07	79.77	85.73	81.62
Oil content	3.49	2.82	3.87	4.45	4.02	2.84	2.69	4.64	3.68	3.94	4.39	4.47	4.69	4.54	4.69	5.74	4.73
C 16:0	15.05	13.21	20.66	16.80	12.92	18.71	11.50	16.06	17.73	13.21	13.74	14.10	14.14	13.54	12.64	14.30	12.85
C 18:0	3.53	2.29	7.32	4.04	1.36	4.45	1.77	1.91	2.90	1.87	1.78	2.35	2.02	1.68	1.51	2.10	1.82
C 18:1	34.30	30.62	34.75	37.42	37.22	35.51	32.96	44.49	41.57	41.84	38.31	41.03	42.73	39.62	38.39	40.96	39.29
C 18:2	43.86	51.67	36.05	39.95	47.22	40.46	51.95	36.27	36.81	42.19	44.58	41.69	40.01	44.02	45.93	41.44	44.48
C 18:3	3.27	2.22	1.22	1.80	1.28	0.87	1.82	1.27	0.99	0.89	1.58	0.83	1.10	1.14	1.53	1.19	1.56
ΣTSEFA	18.57	15.50	27.98	20.84	14.28	23.16	13.27	17.97	20.63	15.08	15.52	16.45	16.16	15.22	14.15	16.40	14.67
ΣTUSFA	81.43	84.50	72.02	79.16	85.72	76.84	86.73	82.03	79.37	84.92	84.48	83.55	83.84	84.78	85.85	83.60	85.33
Oil content	2.67	3.65	4.53	4.01	4.18	3.89	4.28	2.87	3.35	3.35	2.32	3.85	**	0.2083			
C 16:0	13.43	12.13	14.90	14.54	20.64	13.19	13.29	14.12	16.18	13.21	13.11	14.39	**	0.0526			
C 18:0	2.24	2.04	2.28	2.67	5.70	1.73	2.31	1.24	2.59	1.87	1.53	2.63	**	0.0579			
C 18:1	41.14	36.43	41.74	41.38	36.58	36.19	42.43	42.90	42.41	42.41	36.85	39.82	**	0.0721			
C 18:2	41.72	47.46	40.51	40.23	35.06	46.87	40.74	39.63	36.81	42.19	46.74	41.61	**	0.0648			
C 18:3	1.47	1.93	0.58	1.18	2.02	2.02	1.23	2.11	2.01	1.77	1.77	1.55	**	0.0719			
ΣTSEFA	15.68	14.17	17.18	17.21	26.34	14.92	15.59	15.36	18.77	14.64	14.64	14.64					
ΣTUSFA	84.32	85.83	82.82	82.79	73.66	85.08	84.41	84.64	81.23	85.36	85.36	85.36					

16:0: Palmitic acid, **18:0:** Stearic acid, **18:1:** Oleic acid, **18:2:** Linoleic acid, **18:3:** Linolenic acid, **TSFA:** Total saturated fatty acid, **TUSFA:** Total unsaturated fatty acid

The oleic acid content of sorghum genotypes obtained in our work agreed with that reported by Osman et al. (2000) and Mehmood et al. (2008), but was higher than that reported by Cherian et al. (2002), Liu (2011).

Linoleic acid was the predominant component of seed oils of all studied genotypes. Linoleic acid ranged from 29.85 to 51.95%. The highest linoleic acid was found in SL41 genotype, while the lowest percentage of linoleic acid was found in SL25 genotype. Linolenic acid ranged from 0.58 to 5.41%. SL25 genotype (5.41%), SL35 genotype (3.27%), SL31 genotype (3.09%), SL8 genotype (2.84%), SL24 genotype (2.76%), SL36 genotype (2.22%), Rox variety (2.11%), SL56 and SL57 genotypes (2.02%) and Beydari variety (2.01%) have the highest oleic acid composition. The mean values of linoleic and linolenic acids of sorghum genotypes obtained in our work agreed with that reported Osman et al. (2000), Cherian et al. (2002), Mehmood et al. (2008). Liu (2011) stated that linolenic acid content of sorghum seeds were 2.16-2.27% higher than our results belonging to mean values.

Total unsaturated fatty acid (TUSFA) of studied genotypes was between 72.02% and 87.06% (Table 2). From the table presented it could be seen that the highest TUSFA was found in SL7 genotype, while the lowest percentage was found in SL37 genotype. The TUSFA content of sorghum genotypes obtained in our work agreed with that reported by Osman et al. (2000), Cherian et al. (2002), and Mehmood et al. (2008). Total saturated fatty acid (TSFA) of studied genotypes was between 12.94% and 27.98%. SL37 genotype has highest level of TSFA (27.98%); also in the SL31 genotype (27.93%), SL24 genotype (24.51%), SL14 and SL56 genotypes (23.64%), SL 25 genotype (23.62%), SL40 genotype (23.16%), SL38 genotype (20.84%), SL43 genotype (20.63%) and SL32 genotype (20.23%). The lowest percentage of TSFA was found in SL7 genotype. Osman et al. (2000) reported that N.E.S1007 sorghum variety has highest total saturated fatty acid (20.64%). In another study, RARI S-3 sorghum variety (22.46%), DS-97-1 sorghum variety (21.33%) and RARI S-4 sorghum variety (21.23%) have saturated fatty acid concentrations (Mehmood et al. 2008).

Although biplot analysis is generally used in multivariate environmental analysis of genotypes, it is also used in analysis of two-way data. It allows the compari-

son of genotypes with regard to several characteristics (Akcura 2011; Kokten et al. 2012). In current biplot analysis, 83% of total variation in standardized data was explained. Considering the vector lengths of investigated traits, it was observed that oleic acid and linoleic acid had the greatest capacity in separation of genotypes; palmitic acid, stearic acid and linolenic acid had medium-level separation capacity (Figure 1). The size of the angle between the vectors indicates the relationships between the characteristics. Since the cosine of the angle between the vectors of two traits approximately estimates the correlation coefficient between them, this image of the biplot is quite significant in visualizing the relationships among the characters (Akcura and Kokten 2016). It can be said that a positive correlation was observed between palmitic and stearic acid (Figure 1). Besides, the correlation matrix was shown in Table 3 approved Figure 1. Additionally, significant negative correlations were monitored among linolenic acid and

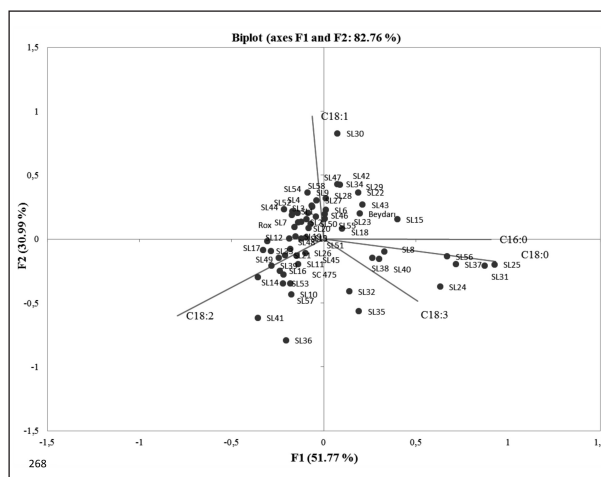


Figure 3. The Biplot of 61 sorghum genotypes for fatty acid composition

Table 3. Correlation matrix showing the relationship between fatty acids

	C16:0	C18:0	C18:1	C18:2	C18:3
C16:0	1				
C18:0	0.821	1			
C18:1	-0.159	-0.251	1		
C18:2	-0.688	-0.615	-0.554	1	
C18:3	0.238	0.455	-0.312	-0.190	1

In bold, significant values (except diagonal) at the level of significance $\alpha=0,050$ (two-tailed test)

palmitic, stearic, oleic acids. Although genotypes exhibited similarities in various characteristics, the genotypes SL36, SL41, SL24, SL31, SL25 and SL30 were different from the others (Figure 1). For breeding studies, these genotypes could be used genitors.

Table 4 shows the eigenvalues and percentages of variance of the PCs created from biplot. Kaiser's rule informs that eigenvalues higher than 1.0 are accepted as descriptors of variance in the data set. The first 2 PCs had eigenvalues higher than 1.0 in our study. PC1, with the highest eigenvalue (2.58), explained 51.77% of the variance in the data set and also PC2 explains 30.99% of variance (eigenvalue=1.55). These two PCs were adequate for qualitative purposes with percentage of 82.76% higher than 70% (Larrigaudiere et al. 2004). Table 5 represents the factor loadings showing the effect of principal components on fatty acids. According to factor loadings, among the fatty acids C16:0, C18:0 and C18:2 explained the variation in PC1 while C18:1 was responsible for the variation of PC2.

Conclusion

In conclusion, palmitic, oleic and linoleic acids were the most abundant fatty acids and stearic and linolenic acids were low in sorghum genotypes. The oil contents of sorghum genotypes showed quantitative differences but the seed oils generally showed uniform

Table 4. Results of the biplot analysis and Eigen values for fatty acids

	F1	F2	F3	F4
Eigenvalue	2,588	1,550	0,716	0,146
% variance	51,770	30,995	14,311	2,925
Cumulative %	51,770	82,764	97,075	100,000

Table 5. Factor loadings showing the effect of principal components on fatty acids

	F1	F2	F3	F4
C16:0	0.908	-0.003	-0.346	-0.234
C18:0	0.933	-0.177	-0.121	0.291
C18:1	-0.061	0.961	0.268	0.030
C18:2	-0.794	-0.599	-0.106	0.024
C18:3	0.510	-0.486	0.706	-0.073

fatty acid compositions. Short-chain fatty acids such as lauric and myristic acids and long-chain eicosenoic and erucic acids are not desired in edible oils. So it could be concluded that seed oil obtained from different genotypes of *Sorghum bicolor* could be alternative source of edible oil due to presence of all saturated and unsaturated fatty acids required for human health and due to absence of such as lauric, myristic, eicosenoic and erucic acids.

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