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Original article

Effects of different irrigations levels on fatty acid components of soybean plant

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Abstract. The aim of this study was to determine the effect of different irrigation levels on fatty acid components of forage soybean. The study was carried out in Kahramanmaras, Turkey. Yesilsoy variety of soybean, which is suitable for growing in semi-arid climatic regions, was used in the study. Four levels of irrigation (I_{100}, I_{75}, I_{50}, I_{25}) were applied to the plants. I_{100}: the treatment in which the water need of the plants was met completely, I_{75}: 75% of the treatment I_{100}, I_{50}: 50% of the treatment I_{100}, I_{25}: 25% of the treatment I_{100}. As a result of the study, the oleic and linoleic acids were found to have the largest proportion in the total fatty acid content. There was no difference between the treatment groups in terms of fatty acids except arachidic acid. The highest arachidic acid content was found to be in the treatments I_{50} and I_{75} with 0.48%, while the lowest in the treatment I_{25} with 0.21%. Significant positive correlations were found to exist between oleic and palmitic acids (r=0.264) and between oleic and behenic acids (r=0.358). This study will contribute to the future studies to be carried out in different locations with more soybean varieties.

Key words: Soybean, irrigation, fatty acids, principle component analysis

Introduction

Soybean is one of the most significiant products cultivated worldwide because it is not only an important resource of vegetable oil for human consumption and industrial use, but also an important resource of vegetable protein for human nutrition and farm animal feed (1). Soybean is a reasonable and benefecial agricultural trade good thanks to its matchless chemical compound. Among grains (cereals) and other legume families, soybean seeds have the highest protein content and a high content of oil (20-25%) (2). Soybean seed compound is one of the important factors of soybean seed quality. Oil and protein density determine the soybean quality because soybean seed is a significant source of rich quality oil and protein (3). The real compound of soya beans depends on different factors such as genotype, cultivation season, geographical position, and agronomic application (4-8). Irrigation (IR) application was also reported to influence the oil and protein components in soybean (9). Several studies showed that negative conditions, particularly drought stress, caused a change in the grain composition and the related quality factors such as oil amount or oil structure. It has been shown that the deficiency of water is a restricting factor for grain growth and affects its compound (10,11,12).

Crop irrigation makes up 70–80% of all water expenditure in arid or semi-arid areas (13,14); thus, the improvement of water management policies for the agricultural areas is a solution in maintaining the presence of water.

The insufficiency of water supply is one of the major difficulties in the world, especially for the main water users, i.e., those in agriculture (15). Considering the water deficiency in the world, deficit irrigation is an effectual farm land strategy that can maintain water, as well as developing product yields in case of water deficiency (16).

In semi-arid regions of the world, soybean is a significant product contributing to the economy; however, water resources are not sufficient in these regions and the irrigation methods used are not suitable. For instance, the water resource is a critical restricting factor for soybean production in semi-arid areas of the Turkey where a great deal of soybean cultivation entirely relies on irrigation (17).

Little or no knowledge is available about the impacts of irrigation on soybean oil. Therefore, the objective of this research was to measure the effect of four levels of irrigation (I_{25}: 25%; I_{50}: 50%; I_{75}: 75%; and I_{100}: 100% field capacity) on the fatty acid composition of soybean grain.

Material and Method

This study was carried out in the research area of the East Mediterranean Transitional Zone Agricultural Research of Institute in the 2019 production season. Typical Mediterranean climate characteristics (mild and rainy winters, hot and dry summers) were observed in the region where the study was conducted. The soil of the field was defined by its sand and loam content in the 0–90 cm bottom range (average 7.45% clay, 50.38% silty, and 42.16% sand). The total available moisture of the soil (90 cm deep) was 138.55 mm.

Yesilsoy soybean cultivar was used as the plant material in this study. Soybean seeds were sown in a dept of approximately 3 cm as a secondary crop. The trials were planned using a randomized complete block design with three replications. The plot size was 17.5 m^2 (3.5 m×5.0 m). The plant–plant spacing was 5 cm, and the row spacing was 0.70 m. The seeds were sown on 22 June 2018 with a pneumatic drilling in 5 rows in each plot. 5 kg N and 7 kg P_2O_5 were applied to the soybean plants along with the sowing. When the plants reached a height of 20-40 cm, 8 kg da^{-1} N 46% urea fertilizer was top-dressed.

Four different irrigation levels (100%, 75%, 50%, and 25%, water consumption relative to the field capacity) were used in the study. The irrigation treatments were determined depending on completing the moisture deficiency in the soil. The irrigation treatments were as follows: full irrigation of 0-90 cm of the soil profile (I_{100}), 75% of I_{100} (I_{75} = 25% water deficit), 50% of I_{100} (I_{50} = 50% water deficit), and 25% of I_{100} (I_{25} = 75% water deficit). Field plot was irrigated by drip irrigation system, and the volume of the water was measured using a water meter. A lateral pipe was laid in each row in the plot. The lateral pipe had a wall thickness of 16 mm. The drippers were spaced 20 cm apart, and the water flow rate was 4 liters per hour.

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

Powder and dirt were removed from the soya seeds, and then they were milled. The method reported by Hara (18) was modified and used; the milled seed samples were homogenized in 5 mL of hexane/isopropanol (3:2), centrifuged at 4500 rpm for 10 min, and filtered. Then, 2.5 mL of 2% methanolic H_2SO_4 was added and vortexed. The mixture was left for 15 h at 50 °C. After 15 h, the tubes were removed and cooled down at room temperature and vortexed by adding 2.5 mL of 5% NaCl. Fatty acid methyl esters (FAME) formed in the tubes were then extracted with 2.5 mL of hexane. The hexane phase was taken with a pasteur pipette and treated with 2.5 mL of 2% Na_2CO_3 and then let for 1 h to separate the phases. The resolvent of the mixture including methyl esters was then evaporated under nitrogen at 45 °C. The upper part was removed and placed in a test tube by filtration. It was dissolved in 1 mL hexane and then measured on GC–MS.

Capillary GLC

The fatty acids in lipid extracts were transformed to methyl esters in methanol with 2% H_2SO_4 (v/v) (19). The FAME were extracted with n-hexane. Then the methyl esters were separated and quantified by gas chromatography with FID (flame ionization detection). Chromatography was performed with a GC-MS equipped with a capillary column [GC–MS instrument (USA) of Agilent brand 7890 A model GC with 5970 C model MS and SGE Analytical BPX90 100m x 250 µm x 0.25 µm column (Australia)] using helium as carrier gas (flow rate 1 mL/min). Hydrogen (H2) and nitrogen (N2) were used for the flame ionization detector (FID).

Other chromatographic parameters were set as follows: from 120 °C to 252 °C with a temperature gradient of 3 °C/min, hold at 120 °C for 8 min, from 120 to 252 °C with a temperature gradient of 3 °C min, hold at 252 ºC for 10 min. Total analysis duration was 62 min. Injection capacity was 1 μL. Individual compounds were identified by frequent comparisons with standart mixes under the same conditions.

Statistical analysis

ANOVA was performed on the data obtained from the experiment. The averages were compared by Duncan grouping. In addition, the mean data obtained from the irrigation levels and fatty acids were subjected to principal component analysis (PCA) biplot analysis using JMP 15.1. (20).

Results and Discussion

In this study, the fatty acid compositions of Yesilsoy soybeanvariety seeds were identified in different levels of irrigation, and the result were given in Table 1. The irrigation levels had no significant effect on fatty acid components except arachidic acid. Gas chromatographic survey of soybean seeds showed that the fatty acid composition was composed of 8 dissimilar fatty acids. Linoleic acid, oleic acid, and palmitic acid were found to be at the highest levels in the seeds. As can be seen in Table 1, there was no statistically significant difference between the irrigation levels in terms of myristic acid. Myristic acid was found to be 0.11% in all the irrigation levels except $I_{25.}$ These results were similar with those (0.058%-0.065%) reported by Sultan et al. (21) under the rainfed conditions.

Palmitic and stearic acids are the most common saturated fatty acids found in vegetable oils (23). Palmitic acid value did not differ between the irrigation levels. The values were close to each other. Palmitic acid values of all the irrigation levels varied between 11.2% and 11.4%. In the literature, palmitic acid value was reported by Abdelghany et al. (24) as 12.12%, Sultan et al. (21) as 9.79, Kırnak et al. (25) as 9.95%-10.61%. The results reported by these searchers were similar to our results. Stearic acid values did not differ statistically depending on the irrigation level. The highest stearic acid value was found to be 1.59% in the treatment I_{25}, while the lowest was 0.76% in the treatment I_{75}. These values were found by Henry (26) as 2.5% and 5.4% and Kırnak et al. (25) as 4.50% and 6.20%, respectively. On the other hand, Abdelghany et al. (24) reported the stearic acid value as 3.94%. Karaca and Aytaç (23) reported that the differences in terms of stearic acid were significant in higher moisture deficiencies, and the contents of fatty acids increased or decreased depending on the level of water deficiency.

As can be seen in Table 1, there was no statistical difference between the irrigation levels in terms of oleic acid. The oleic acid levels were found to range between 33% and 34%, which was higher than those reported by (24) as 21.63%, Sultan et al. (21) as 20.09%, Henry (26) as 17.7%-28%; Kırnak et al. (25) as 26.79%-30.21%. This result made us think that the high oleic acid content in soybeans grown in semi-arid conditions might be an alternative to the oleic acid content in fish oil.

Table 1. Fatty acid compositions (%) by irrigation level

Irrigation levels	Myristic acid (C14:0)	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)	Arachidic acid (C20:0)	Behenic acid (C22:0)
I_{25}	0.10	11.52	1.59	33.10	47.09	6.20	0.21^{b*}	0.15
I_{50}	0.11	11.54	0.89	34.61	46.31	5.83	0.48^{a}	0.20
I_{75}	0.11	11.56	0.76	33.51	47.11	6.16	0.48^{a}	0.26
I_{100}	0.11	11.32	0.84	33.40	47.69	6.14	0.32^{ab}	0.13

* : P≤0.05; The differences between the irrigation levels are significant.

There was no statistically significant difference between the irrigation levels in terms of linoleic and linolenic acids, which are important unsaturated fatty acids like oleic acid. The linoleic acid contents were found to range from 46.31% to 47.69%, while the linoleic acid contents from 5.83% to 6.20%. Linoleic acid content was reported by (24) as 54.41% and Kırnak et al. (25) as 46.23%-48.21%. On the other hand, the linolenic acid contents were reported by (24) as 7.93% and Kırnak et al. (25) as 5.28%-6.22%. The reason why the values obtained the present study were lower than those reported by some researchers is due to the fact that the temperature in the region where this study was conducted was higher than that in the studies of other researchers. Cuniberti et al. (27) reported that as the ratio of linoleic acid and linolenic acid increased, the ratio of oleic acid decreased. This is due to the fact that an increase in temperature causes a decrease in linoleic and linolenic acid contents and an increase in oleic acid content (28).

According to Duncan Test, there were significant differences between the irrigation levels in terms of arachidic acid. The highest arachidic acid content was found to be in I_{50} and I_{75} with 0.48%, while the lowest in I_{25} with 0.21%. These values were reported by Ciabotti et al. (29) as 0.38% and 0.43% and Jokić et al. (30) as 0.35% and 1%, respectively, which are similar to those found in the present study. Behenic acid, which is one of the saturated fatty acids like arachidic acid, was not affected by the irrigation deficiencies. Behenic acid values ranged between 0.13% and 0.26%. The behenic and arachidic acid values reported by Kırnak et al. (25) were lower than 0.4%. Since the arachidic and behenic acid contents have a low share in the total fat composition, not many results were found.

Biplot chart was also created to visually evaluate the relationship between the features. Narrow angle indicates a positive and high correlation between features, and these features are located close to each other (31). The biplot graph of the relationship between irrigation levels and fatty acids is given in Figure 1. This biplot analysis explained 84% of the total variation. The magnitude of the angle between the vectors gives an idea about the relationships between the features. The cosine of the angle between the vectors of two features is crucial for visualizing the link between parameters (32). There was a positive correlation between behenic acid and oleic acid and between palmitic acid and oleic acid (Table 2 and Figure 1). On the other hand, there were significant negative correlations between the linolenic acid and the palmitic and oleic acids (Table 2 and Figure 1). Kaplan et al. (33) reported that they found a negative correlation between the linoleic acid and the palmitic, stearic, and oleic acids in sorghum plant. Table 3 shows the eigenvalues and variance percentages of the Principal Components (PCs). According to Kaiser's rule, eigenvalues higher than 1.0 are accepted as variance descriptors. The first 3 PCs had eigenvalues higher than 1.0 in this research. PC1, with the highest eigenvalue (4.168), explained 52.105% of the difference in the data set and PC2 explained 31.689% of the difference (eigenvalue=2.535). According to (34), it is sufficient for the PC to be greater than 70%.

Table 2. Correlation coefficients showing the relationship between fatty acids

	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0
C14:0	1.000							
C16:0	-0.681	1.000						
C18:0	-0.778	0.184	1.000					
C18:1	-0.074	**0.264**	**-0.477**	1.000				
C18:2	0.616	-0.728	-0.004	**-0.803**	1.000			
C18:3	0.140	-0.213	0.373	-0.989	0.804	1.000		
C20:0	0.298	0.361	-0.829	0.741	-0.517	-0.634	1.000	
C22:0	0.022	0.716	**-0.517**	**0.358**	-0.455	-0.227	0.814	1,000

The significant relationships at the significance level of 0.050 are given in bold.

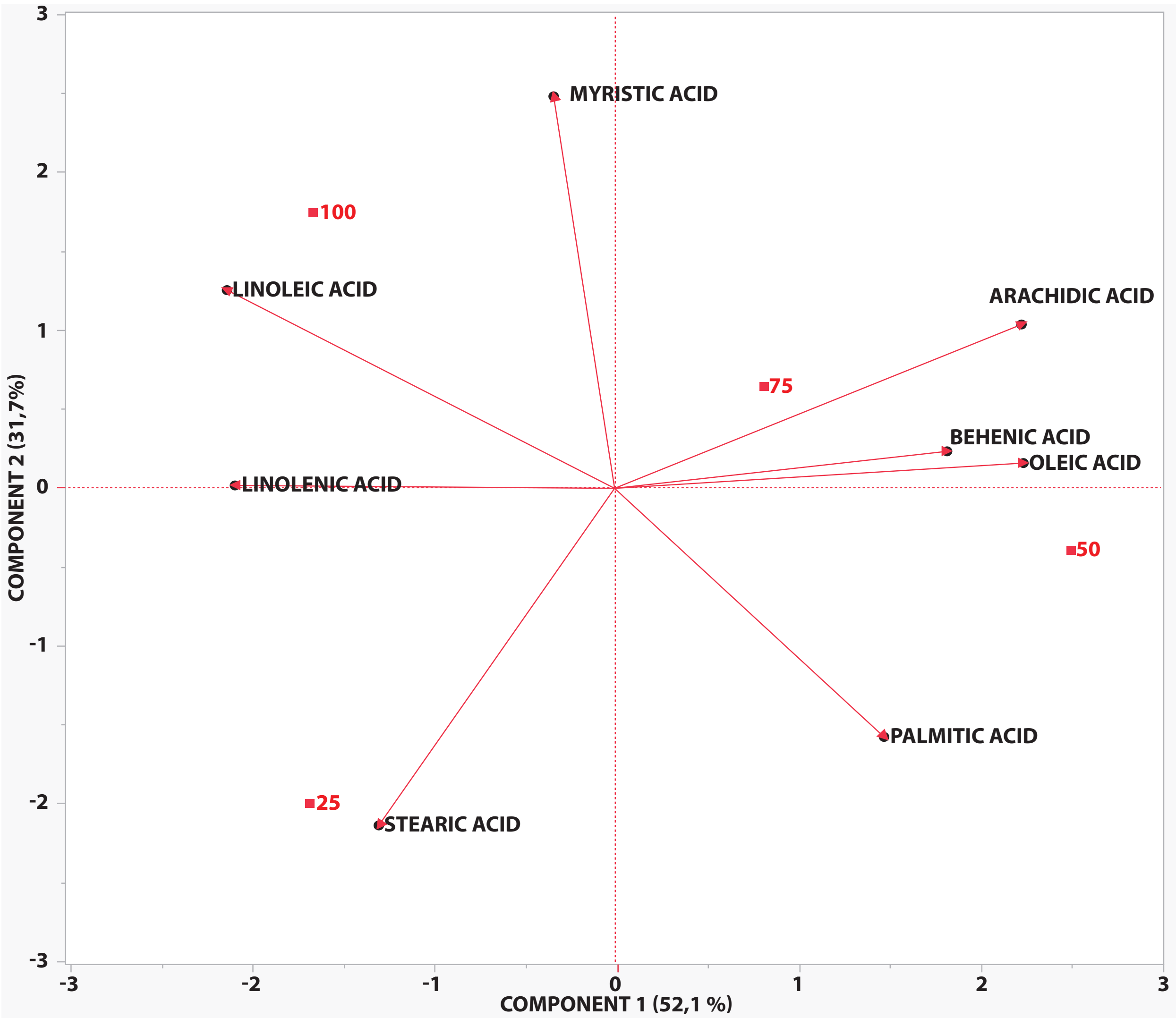

Figure 1. The biplot of 4 irrigation levels for fatty acid composition

Table 3. Eigen values for biplot analysis of fatty acids

	1	2	3
Eigenvalue	4.168	2.535	1.297
Percent (%)	52.105	31.689	16.206
Cum Percent (%)	52.105	83.794	100.00

Conclusion

In conclusion, it was found that the irrigation levels did not cause a statistically significant difference in terms of fatty acids except arachidic acid. The highest arachidic acid content was observed in I_{50} and I_{75} with 0.48%, while the lowest in I_{25} with 0.21%. It was observed that as the water deficit level increased, the amount of stearic acid increased. Significant positive correlations were found to exist between fatty acids, such as between oleic and palmitic acids (r=0.264) and between oleic and behenic acids (r=0.358). We are of the opinion that the oleic acid found in soybean might be an alternative to that found in fish oil. The effect of irrigation levels on fatty acid components of soybean seeds was found to be not statistically significant except arachidic acid. This study will contribute to future studies to be carried out in different locations with more soybean varieties.

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