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PERFORMANCE OF *GLYCINE MAX* (L.) MERR. GENOTYPES UNDER MAIN AND SECOND CROPPING SYSTEMS: II. FATTY ACID COMPOSITION

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Abstract

The impact of planting date on fatty acid composition of soybean genotypes planted at main and second cropping systems was determined. The experiment was conducted in two planting dates in the main plots with four genotypes, namely HA 16-21 (MG IV), HA 36-37 (MG IV), Nova (MG III) and SA-88 (MG III)] in the sub-plots. The results showed that stearic (18 : 0), oleic (18 : 1), alpha (C18 : 3n-3) and gamma-linolenic acid (C18 : 3n-6), eicosadienoic acid (20 : 2) and lignoceric (24 : 0) contents decreased, whereas, linoleic (18 : 2) and arachidic (20 : 0) increased with delay in planting date. Total unsaturated fatty acid content was higher in soybean genotypes planted late. With regard to genotypes, HA 36-37 (MG IV) was high in total unsaturated fatty acid content for both planting dates.

Introduction

Soybean oil contains a high concentration of the polyunsaturated linoleic (18:2) and linolenic (18:3) acids. These fatty acids have a high number of double bonds which are susceptible to oxidation resulting in reduced shelf life, low stability at high cooking temperatures, and off-flavors. Oxidation of linolenic acid with three double bonds is the most important factor contributing to the poor functionality of soybean oil. To improve oxidative stability and undesirable taste, soy oil is hydrogenated to reduce double bonds which are sites of oxidative attack and subsequent off-flavor development (Yadav 1996). Seed composition can be affected by environmental factors such as high air temperature (Gibson and Mullen 1996, Khan *et al.* 2011). High day/night air temperatures and drought conditions during seed filling and maturation affected the fatty acid composition of the soybean seed (Gibson and Mullen 1996). Planting date effects on seed composition are also genotype-dependent (Kumar *et al.* 2006). Several studies reported that higher environmental temperatures reduced linolenic and increased oleic acids concentration of soybean, and indicated that planting date effects had significant impact on fatty acid composition. Previous studies have reported that temperature during seed development is the primary environmental factor governing seed composition (Kane *et al.* 1997, Piper and Boote 1999, Jaureguy *et al.* 2013). Although results may vary with cultivars and planting dates used, the general consensus is that for the same maturity group soybeans, the later the soybean is planted, the more likely the seed-fill period takes place in cooler temperatures than early-planted soybeans (Jaureguy *et al.* 2013).

In southern of Turkey, soybeans are either planted in mid-May as main crop, or in late June as a double crop after small grain winter crops, such as wheat, barley or lentil. The objective of this study was to quantify the impact of planting date on fatty acid of four soybean genotypes planted at main (optimum) and second (late) cropping systems.

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Materials and Methods

The study was conducted in the experimental field of the Department of Field Crops University of Dicle (37°53′N, 40°16′E, 670 m a.s.l.) in southeastern of Anatolian region of Turkey during the 2010 growing season. The region has a warm climate in summer, and the mean annual rainfall is around 450 mm, most of which fall in a major cropping season which extends from November to June. Thus, soybean can be grown during double cropping season with irrigation in cereal or food legume-based cropping systems in the region. Monthly rainfall, mean air temperature and humidity for the growing season are presented in Table 1.

Table 1. Monthly temperature (⁰C), rainfall (mm) and humidity (%) in 2010 growing season (Diyarbakir Turkish State Meteorological Service, 2010).

Months	Air temp. (ºC)			Soil temp. (ºC)	Rainfall (mm)	Humudity (%)
	Min.	Max.	Mean	Mean		
May	7.0	34.1	20.4	21.8	31.6	49
June	11.7	42.0	27.2	29.7	11.2	29
July	17.7	43.3	32.3	34.1	0.0	20
August	17.2	42.7	32.0	34.1	0.4	18
September	13.8	39.7	27.0	28.4	0.0	27
October	3.9	31.0	18.1	18.8	63.0	56

The treatments were replicated three times in split plot based on randomized complete block design with two planting dates (optimal planting date as main crop –20 May, and late planting date as second crop after wheat harvest - 25 June) in the main plots and four genotypes in the sub-plots. Split plots within each main plot had four genotypes: Maturity group (MG) IV genotypes HA 16-21 and HA 36-37 developed by Cukurova University; Maturity group III commercial cultivars Nova and SA 88. After harvesting, seeds were threshed from the pods in a threshing machine, cleaned with the use of sieves. The seed samples were ground, the oil was extracted with diethyl ether using soxhlet apparatus. Fatty acid analysis of the seed oils was carried out in TUBITAK-MAM (The Scientific and Technological Research Council of Turkey-Marmara Research Center). Methyl esters of fatty acids of samples were prepared according to IUPAC II. D. 19. (1979) method and the analysis was held by using Elmer Autosystem XL Gas Chromatography and Flame Ionization Detector (FID). In determining the fatty acids' composition, Supelco 2330 Fused Silica Capillary Column (30 m × 0.25 mm × 0.20 µm film thickness) was used.

The results were expressed as the percentage of each fatty acid with respect to the total fatty acids. All the data are presented as means ± standard error. The statistical significance of differences in the fatty acid composition between groups was analyzed with analysis of variance (ANOVA) and Tukey's HSD test using a statistical software package (JMP version 5.0.1a); $p < 0.05$ was taken to indicate a statistically significant difference.

Results and Discussion

Significant differences among genotypes for all fatty acid components were observed (Table 2). Palmitic acid occurred in 9.78 - 11.03%. Nova had the highest, and HA 16 - 21 the lowest average palmitic acid content although no significant difference was found between this genotype and HA 36 - 37 (Table 3). Stearic acid concentration occurred within 4.47 and 5.53%. The highest stearic acid was obtained from SA 88 (5.53%), while the lowest was obtained from HA 16-

21(4.47%). Oleic acid was detected in 23.27 - 27.42%. The highest oleic acid acquired in HA 36-37 (27.42%), which was statistically at par with the Nova (27.18%) and SA 88 (26.69%), while the lowest values of this trait was recorded from HA 16-21 (23.27%). As a result of negative relation between oleic and linoleic acid, HA 16-21 (52.57%) produced higher linoleic acid compared to the other three genotypes (Table 4). Nova and SA 88 were found to have the highest gamma-linolenic acid (0.468 and 0.462%, respectively) followed by HA 36-37 (0.422), whereas the lowest gamma-linolenic acid content was recorded in HA 16-21 (0.342%). The highest level of alpha-linolenic acid was found in HA 36-37 (0.212%), which is at par with Nova (0.207%) and SA 88 (0.203), whereas, the lowest amount was detected in HA 16-21 (0.192). Genotype HA 16-21 (7.62%) was found to have the highest arachidic acid, followed by SA 88 (5.99%), HA 36-37 (5.91%), and Nova (5.90%) with the least content (Table 5). The highest content of eicosadienoic acid was found in SA 88 (0.410%) and the least was in HA 16-21 genotype (0.343%), the rest two genotypes having intermediate values. SA 88 had the highest lignoceric acid (0.188%) content although there was no significant difference with HA 36-37 (0.172%), whereas, HA 16-21 had the lowest (0.150) content. The highest content (22.40%)of saturated fatty acid was found in Nova, SA 88 (22.03%) and HA 16-21 (22.01%), and the least (21.28%) was in HA 36-37 (Table 6). Unsaturated fatty acid, on the other hand, showed HA 36-37 as the genotype with the highest content (77.37%) and Nova with the lowest (76.02%), with a difference of 1.35% between them. The genotype HA 36-37 had maximum ratio of unsaturated to saturated fatty acids (3.64%). The lowest ratio of unsaturated to saturated fatty acids was observed in HA 16-21, SA 88 and Nova. The highest ratio of oleic to linoleic acid (3.49, 3.48 and 3.39%, respectively) acquired in Nova (0.58%), was statistically at par with HA 36-37 and SA 88 (0.56 and 0.55, respectively), and the lowest values of this trait were recorded from HA 16-21 (0.44%). These results showed that the difference for these components might be due to the genetic variation in varietal characters and different maturity group.

Data statistically analyzed as split plot showed significant planting date main effects for all variables except palmitic (16 : 0) acid (Table 2). According to the results, crops sown optimal planting date (May 20 as main crop) gained higher stearic and oleic acid (5.46 and 28.11%, respectively) compared to late planting date (July 25 as second crop) (4.76 and 24.16%, respectively) (Table 3). The proportion of fatty acids in seed oil is predominantly influenced by enzymes regulating the relation of stearic and oleic exported from the plastids and by enzymes which subsequently metabolise these fatty acids, e.g. oleic by desaturation to linoleic (Hills and Murphy 1991). In the present experiment, the increase of stearic and oleic acid in the early planting might be due to higher temperature during the seed filling stage, especially during August. This is in conformity with Stumpf (1989) who had reported that stearic and oleic acids were enhanced by higher temperatures during the seed filling period. With regard to linoleic acid (Table 4), significant differences were evident between the two planting dates, with a remarkable increase in the late planting (47.74 vs. 51.30%). The higher increase in linoleic acid at late planting might be due to lower temperature coinciding with the seed filling stage from September to October. However, optimal planting date led to increase of alpha-linolenic and gamma-linolenic acid content in four soybean genotypes. It has been suggested that during seed maturation alpha-linolenic acid is transformed into gamma-linolenic acid (Chisowski *et al.* 1993). The result is that early planting is usually associated with an increase in oleic acid (Kane *et al.* 1997) and a decrease in linolenic acid concentrations (Kane *et al.* 1997, Ray *et al.* 1998). From Table 5, it is apparent that the amount of arachidic acid in the seeds planted in late (7.06%) was significantly higher than that of optimal planting (5.64%). However, data showed that optimal planting date, associated with higher temperatures during seed filling period, increased eicosadienoic and lignoceric acid content. Optimal planting date resulted in higher total unsaturated fatty acid and ratio of

Table 2. Analysis of variance for fatty acid composition of soybean genotypes at two planting dates.

Treatment	DF	Fatty acid composition (%) 16:0[a]	18:0	18:1	18:2	18:3n-3	18:3n-6	20:0	20:2	24:0	U[b]	S[c]	U/S[d]	O/L[e]
Planting date	1	NS	**	**	**	*	**	*	*	*	*	*	*	*
Genotype	3	**	**	**	**	**	*	**	**	**	**	**	**	**
Planting date × genotype	3	**	NS	*	**	**	NS	NS	**	NS	**	**	**	**

NS, *, **Correspond to nonsignificant or significance at $p < 0.05$ and 0.01, respectively. [b]U: Unsaturated fatty acids. [c]S: Saturated fatty acid. [d]U/S: Ratio of unsaturated to saturated fatty acids. [e]o/L: Ratio of oleic to linoleic acids.

Table 3. Fatty acid composition of soybean genotypes at two planting dates [palmitic (16:0), stearic (18:0) and oleic (18:1) acid content] (%).

Genotype/	C16:0			C18:0			C18:1		
planting date	Optimal[a]	Late	Mean	Optimal	Late	Mean	Optimal	Late	Mean
HA 16-21	10.09±0.17cd	09.47±0.09e	09.78±0.16c	4.69±0.14	4.24±0.04	4.47±0.12c	24.46±0.47c	22.08±0.12d	23.27±0.57b
HA 36-37	09.91±0.11de	10.25±0.02bcd	10.08±0.09bc	5.44±0.12	4.80±0.04	5.12±0.15b	29.74±0.66a	25.10±0.17c	27.42±1.08a
Nova	11.38±0.13a	10.67±0.19b	11.03±0.19a	5.77±0.06	4.87±0.07	5.32±0.20ab	29.95±0.39a	24.41±0.48c	27.18±1.27a
SA 88	10.05±0.09d	10.59±0.42bc	10.32±0.22b	5.94±0.03	5.13±0.10	5.53±0.18a	28.32±0.16b	25.06±0.66c	26.69±0.79a
Mean	10.36±0.18	10.24±0.17	10.30±0.12	5.46±0.15a	4.76±0.10b	5.11±0.11	28.11±0.69a	24.16±0.41b	26.14±0.57

± : Standard error of mean. [a]Letters that are different for planting date and genotypes are significantly different by Tukey's HSD test ($p > 0.05$).

Table 4. Fatty acid composition of soybean genotypes at two planting dates [linoleic (18:2), alpha-linolenic (18:3n-3) and gamma-linolenic (18:3n-6) acid content] (%).

Genotype/	C18:2			C18:3n-3			C18:3n-6		
planting date	Optimal[a]	Late	Mean	Optimal	Late	Mean	Optimal	Late	Mean
HA 16-21	51.65±0.51b	53.49±0.23a	52.57±0.48a	0.203±0.006	0.180±0.000	0.192±0.006b	0.377±0.003e	0.307±0.003f	0.342±0.015c
HA 36-37	47.15±0.98d	50.77±0.37bc	49.96±0.93b	0.220±0.005	0.203±0.006	0.212±0.005a	0.457±0.014c	0.387±0.006e	0.422±0.017b
Nova	44.34±0.09e	51.26±0.50bc	47.80±1.56b	0.207±0.003	0.207±0.008	0.207±0.004a	0.553±0.017a	0.383±0.013e	0.468±0.039a
SA 88	47.82±0.01d	49.68±1.08c	48.75±0.63b	0.217±0.003	0.190±0.000	0.203±0.006ab	0.497±0.012b	0.427±0.006d	0.462±0.016a
Mean	47.74±0.82b	51.30±0.49a	49.52±0.59	0.212±0.002a	0.195±0.003b	0.203±0.002	0.471±0.020a	0.376±0.013b	0.423±0.015

± : Standard error of mean. [a]Letters that are different for planting date and genotypes are significantly different by Tukey's HSD test ($p > 0.05$).

Table 5. Fatty acid composition of soybean genotypes at two planting dates [arachidic (20:0), eicosadienoic (20:2) and lignoceric (24:0) acid content] (%).

Genotype/	C20:0			C20:2			C24:0		
planting date	Optimal[a]	Late	Mean	Optimal	Late	Mean	Optimal	Late	Mean
HA 16-21	6.74±0.27b	8.50±0.38a	7.62±0.44a	0.360±0.010b	0.327±0.003c	0.343±0.008c	0.167±0.013	0.133±0.003	0.150±0.009c
HA 36-37	5.22±0.06c	6.60±0.13b	5.91±0.31b	0.373±0.003b	0.357±0.013b	0.365±0.007b	0.177±0.006	0.167±0.013	0.172±0.007ab
Nova	5.52±0.01c	6.27±0.14b	5.90±0.18b	0.360±0.000b	0.367±0.006b	0.363±0.003b	0.163±0.003	0.150±0.000	0.157±0.003bc
SA 88	5.10±0.11c	6.89±0.29b	5.99±0.42b	0.443±0.006a	0.377±0.013b	0.410±0.016a	0.213±0.006	0.163±0.003	0.188±0.011a
Mean	5.64±0.20b	7.06±0.28a	6.35±0.22	0.384±0.01 a	0.357±0.007b	0.370±0.006	0.180±0.006a	0.153±0.004b	0.167±0.005

± : Standard error of mean. [a]Letters that are different for planting date and genotypes are significantly different by Tukey's HSD test ($p > 0.05$).

Table 6. Fatty acid composition of soybean genotypes at two planting dates [saturated (S), unsaturated (U) and unsaturated/saturated (U/S) acid ratio] (%).

Genotype/	S			U			U/S		
planting date	Optimal[a]	Late	Mean	Optimal	Late	Mean	Optimal	Late	Mean
HA 16-21	21.68±0.05cd	22.35±0.34ab	22.01±0.21a	77.05±0.02bc	76.38±0.34cd	76.72±0.21b	3.55±0.01bc	3.42±0.06cd	3.49±0.04b
HA 36-37	20.75±0.17e	21.81±0.07bcd	21.28±0.25b	77.93±0.30a	76.81±0.22bc	77.37±0.30a	3.76±0.04a	3.52±0.02bc	3.64±0.05a
Nova	22.84±0.19a	21.97±0.02bc	22.40±0.21a	75.41±0.28e	76.62±0.01bc	76.02±0.29c	3.30±0.04d	3.49±0.01c	3.39±0.04b
SA 88	21.29±0.16de	22.77±0.24a	22.03±0.35a	77.30±0.17ab	75.73±0.42de	76.51±0.40bc	3.63±0.03ab	3.33±0.05d	3.48±0.07b
Mean	21.64±0.24b	22.22±0.14a	21.93±0.15	76.92±0.29a	76.39±0.17b	76.65±0.17	3.56±0.05a	3.44±0.02b	3.50±0.03

± : Standard error of mean. [a]Letters that are different for planting date and genotypes are significantly different by Tukey's HSD test ($p > 0.05$).

unsaturated to saturated fatty acid, but lower saturated fatty acids (Table 6). In general, lower temperatures during seed development normally are associated with more unsaturated oil (Brown *et al.* 1975, Casini *et al.* 2003). Crops planted at optimal date gained higher ratio of oleic to linoleic acid (0.59) compared to late planting date (0.47). Warmer temperatures favor the production of oleic acid rather than linoleic (Ray *et al.* 1993, Bachlava *et al.* 2008). The higher increase in linoleic acid at late planting might be due to lower temperature coinciding with the seed filling stage.

Planting date × genotype interaction effect was significant for palmitic (16 : 0), oleic (18 : 1), linoleic (18 : 2), alpha-linolenic (18 : 3n-3), eicosadienoic (20 : 2), saturated (S), unsaturated (U) and unsaturated/saturated (U/S) acid ratio. However, stearic (18:0), gamma-linolenic (18:3n-6), arachidic (20 : 0), lignoceric (24 : 0) fatty acids were not significantly influenced by planting date × genotype interaction (Table 2). In the optimal planting date, Nova (MG III) had the highest content of palmitic and gamma-linolenic acid. Although no significant effects of planting date × genotype interaction was found on stearic acid in this study, Sa 88 (MG III) had the highest content of stearic and eicosadienoic acid in both planting dates (Table 3). HA 36-37 (MG IV) and Nova (MG III) produced higher oleic acid in optimal planting date. On the other hand, the highest linoleic and arachidic acid were obtained from HA 16-21 (MG IV) when planted late. Nova had the highest saturated fatty acid in optimal planting date, while SA 88 (MG III) and HA 16-21 (MG IV) showed higher performance in late planting for this component. In the optimal planting date, HA 36-37 (MG IV) and SA 88 (MG III) had the highest ratio of unsaturated to saturated fatty acid. HA 36-37 (MG IV) and Nova (MG III) were found to be suitable for O/L acid in optimal planting date for cultivation of soybean under conditions of southeast region of Turkey (Table 7).

Table 7. Fatty acid composition of soybean genotypes at two planting dates (oleic/linoleic acid ratio) (%).

Genotype/ planting date	Oleic/linoleic		
	Optimal[a]	Late	Mean
HA 16-21	0.48±0.01c	0.41±0.00d	0.44±0.01b
HA 36-37	0.63±0.03ab	0.50±0.01c	0.56±0.03a
Nova	0.68±0.01a	0.48±0.01c	0.58±0.04a
SA 88	0.60±0.00b	0.51±0.02c	0.55±0.02a
Mean	0.59±0.02a	0.47±0.01b	0.53±0.01

±: Standard error of mean. [a]Letters that are different for planting date and genotypes are significantly different by Tukey's HSD test ($p > 0.05$).

Significant differences were found among genotypes for all fatty acid components examined. The planting date significantly affected for all variables except palmitic (16 : 0) acid. Planting date × genotype interactive effect was significant for palmitic (16 : 0), oleic (18 : 1), linoleic (18 : 2), alpha-linolenic (18 : 3n-3), eicosadienoic (20 : 2), saturated (S), unsaturated (U) and unsaturated/ saturated (U/S) acid ratio. However, stearic (18 : 0), gamma-linolenic (18 : 3n-6), arachidic (20 : 0), lignoceric (24 : 0) fatty acids were not significantly influenced by planting date × genotype interaction. Taking into account the effects of different planting dates on oleic, unsaturated fatty acid, ratio of unsaturated to saturated and ratio of oleic to linoleic acids it can be concluded that HA 36-37 genotype (MG IV) should be used when planting at an optimal planting date.

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