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THE INFLUENCE OF NITROGEN AND RHIZOBIUM APPLICATıON ON FATTY ACID COMPOSITION OF SOYBEAN [*GLYCINE MAX* (L.) MERR.]

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Abstract

The content of soybean seeds can vary widely and is influenced by the environment and management techniques. An experiment was conducted in Siirt province of Turkey in 2019 to investigate the influences of *Rhizobium japonicum* under various fertilizer nitrogen doses on the fatty acid composition of soybean variety Gapsoy-16 soybean. The experimentation was arranged in split plot design with five different nitrogen doses (0 kg/ha, 50 kg/ha, 100 kg/ha, 150 kg/ha, and 200 kg/ha in pure form) as main plots treatments and Rhizobium inoculation (with or without bacteria) as the sub-plot treatments. Results showed that higher nitrogen dosage (200 kg/ha) with and without rhizobium inoculation reduced linoleic and linolenic acids contents but increased oil ratio, oleic, arachidic, stearic and palmitic acids contents of soybean seeds. The determined content of oil ratio, oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20), stearic acid (C18) and palmitic acid (C16) were between 14,8-19,03%; 29,73-31,36%; 31,16-33,06%; 7,32-7,82%; 1,33-1,51%; 3,52-3,86% and 14,91-15,78%, respectively. *Rhizobium* inoculation increased the oil ratio without nitrogen ($N_{1=}$0 kg N/ha) and with adding 200 kg N/ha (N_5) dosage, whereas it reduced the ratio at other N doses

Key words: Fatty acid composition, Fertilizer, Oleic acid, Rhizobium, Soybean.

Introduction

Among the legumes, soybean (*Glycine max* L.) is a very essential recognized oil seed and protein crop worldwide. It is a significant necessary commodity on the current world market (Jia *et al*., 2020). Soybean being an economically essential food and oilseed crop, is a main source of proteins and oils (Lui *et al*., 2020), it is an important legume containing 20% oil (Sharma & Goyal, 2015).

The aim of changes of fatty acid in soybean to enhance oil quality is essential in soybean search for fitting needs of nutritional criteria (Pham, 2011). There is increasing interest in expanding the productivity of oils soybean to face the rise in feed demand (Zhao *et al*., 2021). The American Heart Association recommends 1:1:1 saturated, monounsaturated, and polyunsaturated fatty acid balance contributed by all fats in our daily diet (Rudzinska *et al*., 2018). As a feed source, soybean oil is widely used in freshwater aqua-feeds in countries (China Li *et al*., 2016). It is a necessary raw material for vegan and lactose-free goods (Preece *et al*., 2017).

Nitrogen, an important macronutrient, is needed by plants in the largest quantity and is the principal constraining factor for plant development (Samudin & Kuswantoro, 2018). The root nodule symbiosis and biological nitrogen fixation in soybeans have critical importance in agriculture due to the symbiosis with *Bradyrhizobium*, which are beneficial of economically and ecologically side due to decrease the need addition for synthetic nitrogen application (Chang *et al*., 2015; Yuan *et al*., 2016) however, the agronomy practices may influence on the effectivity of biological nitrogen fixation (Schmidt *et al*., 2017).

The scientific literature comprehensively reviews the relationships between soybean biological N2 fixation and fertilizer nitrogen for yield response. Keeping in view the nutritional considerations of soybean provide additional economic and ecological benefits so this study objective is to examine the effects of *Rhizobium japonicum* under varied nitrogen fertilizer concentrations on the fatty acid content of a particular soybean variety, Soybean Gapsoy-16.

Material and Methods

The experimental field was conducted at the University of Siirt, Turkey, in 2019 using the Gapsoy-16 soybean variety. The study was arranged in a split-plot design with five different nitrogen doses (0 kg/ha, 50 kg/ha, 100 kg/ha, 150 kg/ha, and 200 kg/ha in püre form) as main plot treatments and *Rhizobium* inoculation (with or without bacteria) as subplot treatments. Each treatment was replicated thrice. The nitrogen fertilizer was applied as a starter dose of urea (46% N).

The experimental area is in the Menzilat soil series (Dingil *et al*., 2008), the experimental soil was slightly alkaline with a clayey structure, low in lime, organic matter, and phosphorus, whereas the potassium content was sufficient in the soil (Table 1). The weather parameters such as temperature, precipitation, and relative humidity recorded during the experimental period are presented in Table 2. The analysis of climatic variables showed that the temperature average in the June–October period of the research year was observed to be similar to long-term averages. The total amount of precipitation received between June–October 2019 was 47,9 mm, which was lower than the precipitation received by the same area for many years from June–October (70,9 mm).

Sowing soybean seeds with a seeder was collected in May after the wheat harvest. For bacterial treating plots, seeds were inoculated with *Rhizobium japonicum* before sowing. Bacteria-untreated plots were seeded just before bacteria-treated plots. *Rhizobium* bacteria were obtained from “Soil Fertilizer and Water Resources Central Research Institute” (Ankara, Turkey). To ensure better adhesion of the bacteria to the seeds, a 4% sugar solution was mixed with bacteria preparation, and the seeds were kept in the shade for 8 hours before sowing. The crop was raised as per the standard package of practices. Earthing up was performed approximately one month after sowing. The crop was irrigated timely with drip irrigation, and no pesticides were applied. The crop was harvested in October at seed maturity by discarding 50 cm row length from both ends to eliminate the border effect. Subsequently, the treatment-wise threshing was done with a threshing machine for collection of seeds.

Table 1. Soil properties of the field trial area.

Analyzes	0-30 cm Depth soil analysis		30-60 cm Depth soil analysis	
	Result	Evaluation	Result	Evaluation
pH	7,12	Notr	7,72	Slightly basic
Total salt (dS/m)	0,11	Salt-free	0,10	Salt-free
Calcium Carbonate (%)	2,73	Slightly calcerous	3,20	Slightly calcerous
Soil texture (%)	Clay: 47,24 Silt: 18,26 Sand: 34,40	Clayey	Clay:47,24 Silt:17,28 Sand:35,48	Clayey
Organic Matter (%)	1,48	Low	1,16	Low
Available Phosphorus (kg/ha)	47,4	Low	0,96	Very low
Available Potassium (kg/ha)	826,7	Sufficient	46,3	Sufficient
Available Calcium (kg/ha)	6980,5		11294,4	
Available Magnesium (kg/ha)	1216,8		121,4	
Zinc (ppm)	0,54		0,36	
Manganese (ppm)	35,04		27,5	
Iron (ppm)	10,88		9,33	
Copper (ppm)	1,73		1,38	
Boron (ppm)	0,03		0,02	

Table 2. Climatic characteristics of Siirt province for 2019 and long years (MGM, 2020).

Months	Monthly average temperature (°C)		Monthly average relative humidity (%)		Monthly average rainfall (mm)	
	2019	Long Years (Average)	2019	Long Years (Average)	2019	Long Years (Average)
June	29.1	25.9	26.5	28.7	1.2	9.3
July	30.2	30.6	23.0	18.1	0.0	2.7
August	30.5	30.3	19.50	17.2	2.6	1.7
September	25.6	25.4	30.0	24.0	12.5	6.9
October	18.1	18.2	47.0	45.3	31.6	50.3

Methods used for assessment of the fatty acid composition: The method used was the esterification method by taking 0,8 ml of oil in the tubes. 1,6 mL 1M KOH (dissolved in methanol) and 2,5 mL hexane were added to it and vortexed. The upper phase formed in the tube was taken into the vial with the help of a syringe and 0,22 PVDF filter was taken to the GC-MS and CS-MS analysis. Fatty acid compositions of seeds were measured with a gas chromatography and the analyses were performed at the central laboratories of Tubitak-Mam (Marmara Research Center) according to the method described by Slover and Lanza (1979).

GC-MS conditions: The process of analysis was performed in a Trace 1310 gas chromatograph processed with an ISQ single quadrupole mass spectrometer (Thermo Fisher Scicentific, Austin, TX). The process was set to 140°C for 5 min, then ramp at 2°C/min to 190°C, hold 190°C for 1 min then ramp at 3°C/min to 240°C and at last 35 min in 240°C. The ion source and detector temperature was 250 °C. Sample was Separated and subjected on a Thermo TG-WAXMS GC column (60 m x 0.25 mm ID x 0.25 µm) thorough helium as carrier gas at 1.2 ml/min. Peak designation was done by comparison of the known traits stored in the NIST Demo, Wiley7, Wiley9, redlip, mainlip, WinRI. Split Ratio 1:60.

CS-MS conditions: GC-MS analysis was done in gas chromatograph equipped with an ISQ single quadrupole mass spectrometer. The ion source and detector temperature were 250°C. 1 mL of sample was dissolved in 5 mL of 100 % $CHCl_3$ and filtered with a 0.22 µm disposable syringe filter. A volume of 1 µL was injected in spitless model. The retention indexes (RI) were described according to (Adam, 2007).

The analyses of data were analysed using analysis of variance (ANOVA) in accordance with split plot analysis experimental design by using the MSTAT-C statistical package programme. Differences between means were compared by Least Significant Difference (LSD) at a level of $p<0.01$, using statistical procedures recommended by (Gomez & Gomez , 1984).

Results and Discussion

In the experiment conducted, oil ratio in soybean seeds was lowest (14,8%) at "N_4 (150 kg/ha) +B" application whereas it was highest (19,03%) at "N_5 (200 kg/ha) +B" application (Table 3). Rhizobium inoculation increased oil ratio without nitrogen application (N_1 = 0 kg/ha) and recorded the maximum value with highest nitrogen application (N_5 =200 kg/ha) whereas the other N doses reduced the oil ratio significantly. It was found that, an increase in oil ratio is generally correlated with a reduction in protein content (Roche *et al*., 2006).

Table 3. Fatty acid contents under different application conditions obtained in the study.

Application	Oil Ratio (%)	Oleic acid (C18:1) (%)	Linoleic acid (C18:2) (%)	Linolenic acid (C18:3) (%)	Arachidic acid (C20) (%)	Stearic acid (C18) (%)	Palmitic acid (C16) (%)
N_1 (0 kg/ha) + B	17.35 c	30,65 c	32,95 c	7,62 e	1,36 h	3,58 ı	15,12 h
N_1 (0 kg/ha) -B	16,47 e	29,73 ı	33,06 a	7,66 c	1,42 f	3,69 f	15,03 ı
N_2 (50 kg/ha) +B	15,04 h	29,8 h	32,51 d	7,68 b	1,46 b	3,73 e	15,14 g
N_2 (50 kg/ha) -B	15,94 f	31,01 b	33,05 b	7,82 a	1,33 ı	3,52 j	14,91 j
N_3 (100 kg/ha) +B	15,04 h	31,36 a	31,87 f	7,34 ı	1,45 c	3,61 h	15,43 f
N_3 (100 kg/ha) -B	17,33 d	29,81 g	31,16 ı	7,41 h	1,45 c	3,86 a	15,78 a
N_4 (150 kg/ha) +B	14,8 ı	30,39 d	30,95 j	7,32 j	1,51 a	3,85 b	15,53 e
N_4 (150 kg/ha) -B	15,13 g	30,08 f	31,38 g	7,53 f	1,44 d	3,8 c	15,61 d
N_5 (200 kg/ha) +B	19,03 a	30,21 e	31,3 h	7,64 d	1,41 g	3,76 d	15,77 b
N_5 (200 kg/ha) -B	18,39 b	30,65 c	32,06 e	7,5 g	1,43 e	3,64 g	15,63 c
Mean	16,45	30,37	32,03	7,55	1,43	3,7	15,4
CV	0,07	0,007	0,01	0,03	0,22	0,05	0,02

*The difference between the means with the same letter is not statistically significant

B- : Without *Rhizobium*; B+ : With *Rhizobium*

Oleic acid (C18:1) content in soybean seeds was lowest (29,73%) without N and *Rhizobium* application (N_1 = 0 kg/ha –B) whereas was highest content (31,36%) found with 100 kg N/ha application + *Rhizobium* inoculation (Table 3). Application of 100 kg N/ha without *Rhizobium* inoculation recorded lowest content (31,16%) of Linoleic acid (C18:2), however the content was highest in seeds (33,06%)) without the application of N and *Rhizobium* (Table 3). Linolenic acid (C18:3) content was lowest (7,32%) with 150 kg N/ha) + Bacterial application, whereas the content was found highest (7,82%) when nitrogen was applied @ 50 kg/ha (N_2) without *Rhizobium* inoculation (Table 3). Application of nitrogen @ 50 kg/ha (N_2) without the seed inoculation recorded lowest content of Arachidic acid (C20) (1,33%), Stearic acid (C18) (3,52%) and Palmitic acid (C16) (14,91%), respectively. at "N2 (50 kg/ha) -B" application whereas the highest content (1,51 %) of Arachidic acid (C20) was observed when 150 kg N/ha (N_4) was applied in combination with *Rhizobium* inoculation. However, the highest content of Stearic acid (3,86%) and Palmitic acid (15,78%) were recorded with application of 100 kg N/ha (N_3) without the inoculation of seeds with *Rhizobium* (Table 3). Luís *et al*., (2013) fount that that Rhizobium inoculation improves the organic and fatty acids ratio of soybean seeds (Dashti1 *et al*., 1997) found that addition of plant growth-improving rhizobacteria to soybean increase oil content. Soybeans with high rates of monounsaturated fatty acids are preferable for consumption of human than saturated fatty acids. The hydrogenation procedure was found to have undesirable health influences by the increase the risk of coronary heart disease and leading to higher LDL-cholesterol and lower HDL-cholesterol (FR, 2003). The inoculation with *Rhizobium* improves unsaturated fatty acids content of soybean seeds was improved (Luís *et al*., 2013; Sharifi *et al*., 2016).

The quality characteristics of Soybean seed in reaction to seven different indigenous *Bradyrhizobium* inoculation and three different N fertilizations under conditions of field were determined in Kashmir, Pakistan and it was found that *Bradyrhizobium* inoculation and nitrogen fertilizer positively improved oil percent compared to the un-inoculated treatment (Rahim *et al*., 2015). The seed oil content changed between 16.2 and 21.5%, mainly composed of linoleic acid (47%) and oleic acid (24%). *Bradyrhizobium* inoculation and N application was caused a reduction in saturated fatty acids (palmitic and stearic) and improved unsaturated fatty acids (linoleic acid and oleic acid). Also, our finding confers that *Rhizobium* application improved oil ratio without nitrogen (N_1 = 0 kg/ha) and with higher nitrogen application (N_5 =200 kg/ha). These variations might be due to different agronomic practices like, no phosphorous application was done to the crop in our experiment.

The experiment further supports the results (Szpunar-Krok *et al*., 2021) at South-East Poland two soybean cultivars were assessed for fatty acid composition under varied nitrogen fertilization and *B. japonicum* inoculation and confirmed fatty acid composition variance accumulation rates for C16:0, C16:1, C18:1n9, C18:2, C18:3, and C20:0 like, saturated, monounsaturated, and polyunsaturated fatty acids. The increase of addition level from 30 to 60 kg ha^{-1} N did not achieve the expected aims and suggested adding only a starter dose of 30 kg ha^{-1} N. But the inoculation of soybean seeds with a strain of *Bradyrhizobium japonicum* is recommended as it will cause a reduction in saturated fatty acids and C16:0 acid rates which is nutritionally advantageous as its contribution to total fatty acids. The increase in the value of C16:0 content leading a reduction in the accumulation of C18:1, C18:2, and C18:3 acids. The results of our study also suggest that *Rhizobium* application increased oil ratio without nitrogen (N_1 = 0 kg/ha) and with higher nitrogen application (N_5 =200 kg/ha). İncreasing N rates or *Rhizobium* applications resulted with mixed and irregular changes for different fatty acid compositions in our study. Generally, Inoculation enhances productivity and fatty acid content in soybean (Igiehon *et al*, 2021), and it also leading an increase in the FA content in seeds (Silva *et al*., 2013).

Conclusions

Based on the experiment's findings, it can be said that Rhizobium inoculation and nitrogen administration greatly changed the fatty acid content of soybean. When soybean seedlings were given a higher nitrogen dosage (200 kg/ha), both with and without Rhizobium inoculation, the amount of linoleic and linolenic acids was reduced, but the amount of oleic, arachidic, stearic, and palmitic acids was raised. Furthermore, it was discovered that Rhizobium inoculation raised the oil ratio in the presence of no nitrogen (N1=0 kg N/ha) and 200 kg N/ha (N5) dosages while decreasing the ratio in the presence of other N doses.

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Conflicts of Interest: The authors declare no conflict of interest.

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