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Original Research

Nitrogen Application and Seed Inoculation with *Bradyrhizobium japonicum* Bacteria Improved Yield and Quality Traits of Soybean [*Glycine max* (L.) Merrill] Grown as Second Crop

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Abstract

Nitrogen-fixing bacteria are essential for providing nitrogen (N) to soybean (*Glycine max* (L.) Merrill) plants, often resulting in a reduced or eliminated requirement for N-fertilizer. The application of these microorganisms can enhance N fixation and maximize soybean yield. Soybean is attaining significant attention in southeastern Anatolia as a second crop; however, the interactive effects of N-fixing bacteria and N doses on its yield are still unknown. This study investigated the effects of N application and seed inoculation with *Bradyrhizobium japonicum* bacteria on yield and quality attributes of soybean grown as a second crop during 2021 and 2022. Seed inoculation (bacteria-inoculated or non-inoculated) were the main plots, whereas N doses [0 (N_0), 40 (N_1), 80 (N_2), 120 (N_3), 160 (N_4), 200 (N_5), 240 (N_6), and 280 (N_7) kg ha^{-1} were sub plots. Bacteria inoculation and N doses significantly ($p \leq 0.01$) altered the number of pods per plant, plant height, thousand seed weight, grain yield, protein ratio, and oil ratio. The highest (3645 kg ha^{-1}) and the lowest (3072 kg ha^{-1}) grain yield was recorded for bacteria-inoculated and non-inoculated seeds, respectively. Similarly, N_7 (3628 kg ha^{-1}) and N_0 (2791 kg ha^{-1}) produced the highest and the lowest grain yield, respectively. Regression analysis revealed that 144 kg ha^{-1} pure N should be applied for the cultivation of the second crop soybean under the climatic conditions of Şanlıurfa, Türkiye, and seeds should be inoculated with *B. japonicum* for better yield and economic returns.

Keywords: soybean, second crop, bacteria, inoculation, nitrogen

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Introduction

Soybean [*Glycine max* (L.) Merrill] is a significant agricultural crop on a global scale [1, 2]. It is regarded as one of the most significant oilseed crops globally due to its extensive cultivation area [3], substantial production volume, and significant involvement in international trade. Soybean covers > 50% of global oilseeds' cultivation area and has a significant contribution as a feed source for livestock such as poultry, meat, and dairy farming [4].

Soybean seeds contain significant amounts of oil, protein, carbohydrates, and minerals, ranging from 18–24%, 35–42%, 30%, and 5%, respectively [5]. Additionally, seeds are rich in vitamins and amino acids. Soybeans provide organic matter and nitrogen (N) to the soil [6]. Crude protein content in the pulp renders soybean as an essential source of nutrition for livestock, while its fat and protein content make it a crucial component in human dietary intake [7, 8]. Approximately 85% of the global soybean production is utilized for manufacturing soybean oil and meals [2].

Soybean is cultivated as a main and second crop in Türkiye [7] and is mostly used in poultry nutrition [9]. Soybean produced in Türkiye cannot fulfill the domestic demands; therefore, significant amount is imported every year [10]. Soybean was produced on an area of 38.009 ha during 2022 in Türkiye, which produced 155.000 tons of grains with an average yield of 4080 kg ha^{-1} [11]. The adoption of monoculture in numerous regions of the country has decreased crop productivity [12]. Excessive fertilization to improve yield increases input costs, reduces farmers' profit, and exerts adverse effects on soil biological productivity [13]. Furthermore, nutrient accumulation in plants and contamination of drinking water have significant implications for human and animal health.

Soybean can fix atmospheric N to the soil due to the presence of *Bradyrhizobium japonicum* bacteria in its root system [14]. Soybean fulfills its N requirements while enriching the soil with N for subsequent plant cultivation. Furthermore, easy decomposition of soybean stalks increases soil organic matter content. For these reasons, soybeans are an ideal candidate for crop rotation [15]. Legumes are unable to utilize atmospheric N on their own. *Rhizobium* bacteria residing in the roots of legume crops facilitate N-incorporation. Therefore, the presence of *Rhizobium* bacteria in the soil is necessary to fix atmospheric N. *Bradyrhizobium japonicum* is not naturally present in Turkish soils; therefore, it is necessary to inoculate seeds with bacteria culture prior to planting soybean in the country.

Kuntyastuti [16] applied 30, 60, 90, 120, 150, 180, and 210 kg ha^{-1} N to soybean in Malang, Indonesia, and the highest (3210 kg ha^{-1}) yield was recorded under 120 kg ha^{-1} N. It has been reported that N application increases plant height and positively affects protein content. Kılınç and Arıoğlu [17] reported that the highest yield (3745.7 kg ha^{-1}) of second crop soybean was obtained with the application of 180 kg ha^{-1} N in Çukurova region, Türkiye. Conversely, the lowest grain yield (3059.3 kg ha^{-1}) was recorded with no N application. Arıoğlu et al. [18] investigated the effects of *B. japonicum* inoculation with different methods on the yield and quality of soybean cultivated as a second crop in the Çukurova region. Three different inoculation methods (i.e., pre-packaging inoculation and pre-sowing powder and liquid bacteria) were used to inoculate soybean seeds. Grain yield, 1000-grain weight, number of pods per plant, plant height, and protein content of bacteria inoculated seeds were higher compared to non-inoculated ones. Seed inoculation with liquid and powder bacterial before sowing proved better than pre-packaging seed inoculation.

Soybean cultivation areas have been increasing in Şanlıurfa province nowadays. It is vital to sow the bacteria-inoculated soybean seeds in the moist soil with the appropriate N dose for obtaining a high yield in the hot season. Although some studies have inferred the role of *B. japonicum* in improving soybean yield and optimized N doses for some regions in Türkiye, no studies have been conducted to optimize N dose for soybean grown as a second crop in southeastern Anatolia. Furthermore, the role of *B. japonicum* bacteria in lowering N requirements for the crop has never been tested in the region.

The present study was aimed at investigating the impact of seed inoculation with liquid bacteria culture commonly used in soybean and different N doses on yield and some quality traits of soybean grown as a second crop in Şanlıurfa, Türkiye. It was hypothesized that seed inoculation with *B. japonicum* would improve the yield and quality attributes of soybean. It was further hypothesized that seed inoculation with *B. japonicum* will lower the N requirements of the crop. The results will help to optimize the N dose for second crop soybeans in the region to get better economic returns.

Materials and Methods

Experimental Site

The experiments were conducted at Talat Demirören Research Station affiliated with the GAP Agricultural Research Institute, Şanlıurfa Province, Türkiye, during 2021 and 2022. Şanlıurfa has a continental climate of the Southeastern Anatolia region, with a minor influence of Mediterranean climate. Summers are arid and hot, while winters are relatively mild. The region is predominantly affected by a dry and hot tropical air mass that settles over the Basra low-pressure center during the summer season. Weather data of the experimental site for the study years are given in Table 1.

Soil samples were collected from 0–30 cm and 30–60 cm depth from the experimental site during both years of the study to determine the soil properties (pH, EC, calcium carbonate, phosphorus, potassium, and organic matter) and fertility status. The collected soil samples were analyzed at the Soil Science Laboratory of the GAP Agricultural Research Institute. Organic matter was determined by the Walkley and Black method. Soil pH and electrical conductivity (EC) were measured

Table 1. Weather data of the experimental site during the growing season of 2021 and 2022.

Months	Soil temperature (°C)				Air temperature (°C)						Relative humidity (%)	
	2021		2022		2021			2022			2021	2022
	10 cm	20 cm	10 cm	20 cm	Max.	Min.	Mean.	Max.	Min.	Mean.		
June	45.1	36.2	36.7	29.5	40.6	11.5	25.8	41.0	12.9	26.7	34.8	42.7
July	45.6	36.7	34.7	29.3	41.7	14.6	29.6	40.7	15.0	28.9	37.4	42.9
August	42.2	35.2	34.9	29.6	40.8	14.0	27.3	40.4	13.6	26.9	50.5	62.5
September	35.8	31.1	34.5	29.7	36.5	8.0	22.1	38.2	8.1	22.7	54.3	60.4
October	27.6	25.0	27.9	24.8	32.4	6.2	20.3	34.2	1.6	17.6	55.6	57.5

Table 2. Physicho-chemical properties of experimental site during study years.

Year	Depth (cm)	Field capacity (%)	pH	EC ds/m	Calcium carbonate (%)	Phosphorus P_2O_5 (kg ha^{-1})	Potassium K_2O (kg ha^{-1})	Organic matter (%)
2021	0–30	63	7.36	0.96	32.6	26.3	888	1.39
	30–60	66	7.42	1.00	31.1	30.4	972	1.25
2022	0–30	64	7.76	1.03	24.3	12.3	639	1.35
	30–60	66	7.81	0.97	25.0	9.8	474	0.94

in a saturated paste. The $CaCO_3$ was determined by the calcimeter method. Potassium was determined by extraction with neutral 1M ammonium acetate, whereas phosphorus was determined by the Olsen method. The soil properties and nutrient availability are given in Table 2.

Treatments

Seeds of the 'Gapsoy 16' soybean variety were procured from the GAP Agricultural Research Institute, Şanlıurfa, Türkiye, and used in the experiment. Liquid bacterial culture (1×10^8 CFU *B. japonicum*) and N fertilizer were procured from the local market. The experiment consisted of two factors, i.e., bacteria inoculation and N doses. Two bacteria inoculation treatments (bacteria-inoculated and non-inoculated seeds) and 8 N doses (0, 40, 80, 120, 160, 200, 240, 280 kg ha^{-1} N) were included in the study (Table 3).

Seeds were inoculated prior to sowing by homogenously mixing the seeds and bacteria culture. Seeds were sown with an experimental drill at a depth of 4–5 cm, 70 cm row spacing, and 3–4 cm plant spacing on June 15th every year. Phosphorus fertilizer (60 kg ha^{-1}) was uniformly applied to all plots, whereas N was applied according to the treatments. Potassium was not applied as it was not required according to soil analysis. The experiments were laid out according to a randomized complete block design with split-plot arrangements and four replications. Bacteria inoculation treatments were kept in main plots, whereas N doses were randomized in sub plots. An area of 2.8 m between experimental plots and 3 m between replications was left blank to prevent N movement. Insecticides were applied when necessary to protect crops from pest infestation. Experimental fields were irrigated with the basin irrigation method, and a total of 900 and 850 mm of water were applied to the crop during 2021 and 2022, respectively.

Data Collection

Ten plants were randomly selected from each experimental plot in each replication to record data on morphological attributes. Plant height of ten randomly selected plants in each replication was measured and averaged. The number of pods present on randomly selected ten plants was counted and averaged. The crop was manually harvested on October 28, 2021, and October 17, 2022. A 0.5 m area was left as a border effect from all sides of the plots, and the remaining area was harvested. A harvester machine was utilized to separate the grains from the husk. Three random samples of 1000 grains were taken from each seed lot, weighed, and averaged to record 1000 grain weight. The harvested grains from each experimental unit were weighed and converted to kg per hectare by the unitary method. Standard official protocols were used to determine seed protein content and oil concentration.

Statistical Analysis

The collected data on morphological, yield-related, and quality traits were analyzed by Analysis of Variance (ANOVA). The differences among years were tested by a paired t test, which denoted no differences. Hence,

Table 3. Bacteria inoculation and nitrogen doses used in the study.

Nitrogen Dose	Bacteria inoculation	Nitrogen at sowing (kg ha^{-1} N)	Nitrogen after sowing (kg ha^{-1} N)	Total Nitrogen (kg ha^{-1} N)
N_0	Inoculated	0	0	0
N_1	Inoculated	40	0	40
N_2	Inoculated	40	40	80
N_3	Inoculated	40	80	120
N_4	Inoculated	40	120	160
N_5	Inoculated	40	160	200
N_6	Inoculated	40	200	240
N_7	Inoculated	40	240	280
N_0	Non-inoculated	0	0	0
N_1	Non-inoculated	40	0	40
N_2	Non-inoculated	40	40	80
N_3	Non-inoculated	40	80	120
N_4	Non-inoculated	40	120	160
N_5	Non-inoculated	40	160	200
N_6	Non-inoculated	40	200	240
N_7	Non-inoculated	40	240	280

Table 4. The grain yield of bacteria inoculated soybean predicted by regression analysis.

Nitrogen doses (kg da^{-1})	N_0	N_1	N_2	N_3	N_4	N_5	N_6	N_7
Grain yield (kg ha^{-1}) (kg da^{-1})	2983	3189	3524	3855	3859	3894	3922	3932

the data of both years were pooled for analysis. A two-way ANOVA was used to infer the significance of the data. The normality in the dataset was tested prior to ANOVA by the Shapiro-Wilk normality test, which indicated a normal distribution. Therefore, original data were used in the analysis. A least significant difference (LSD) post-hoc test at 95% probability was used to compare the means, where ANOVA denoted significant differences. All statistical computations were done on SPSS statistical software version 21.0. Linear regression analysis was conducted between N doses and grain yield to determine the optimum N doses. The linear regression was performed on the Microsoft Excel application of Office 365.

Results and Discussion

Number of Pods per Plant

Individual and interactive effects of bacteria inoculation and N doses significantly ($p \leq 0.01$) affected the number of pods per plant. The highest (87.1) and the lowest (82.6) number of pods per plant were recorded for bacteria-inoculated and non-inoculated seeds, respectively. Similarly, the highest (86.4) and the lowest (83.1) number of pods per plant were noted from the plants receiving N_5 and N_1 doses, respectively. Regarding bacteria inoculation by N doses interaction, bacteria-inoculated seeds×N_7 dose resulted in the highest (89.3), while non-inoculated seeds×N_0 dose resulted in the lowest (80.7) number of pods per plant (Table 5). The results revealed that seed inoculation with liquid bacteria culture and nitrogen fertilization positively affected the number of pods per plant.

The quantity of pods per plant is substantially influenced by growth seasons. Soybean requires shorter day length to flower and reach maturity. Consequently, the duration of vegetative and reproductive stages is shortened under these circumstances. The number of pods per plant is a crucial agronomic characteristic and positively related to soybean yield [19]. Liquid bacterial application resulted in the largest number of pods per plant (75.6 pods plant^{-1}), whereas non-inoculated treatment resulted in the lowest pod count (67.9 pods plant^{-1}) in the Çukurova region [18]. Özkul [20] reported that bacterial inoculation and N fertilization had a beneficial impact on pod count in second-crop soybean cultivation in the Çukurova region. These findings align with our results.

Plant Height (cm)

Plant height was significantly ($p \leq 0.01$) influenced by individual effects of bacteria inoculation and N doses. However, the interactive effect of bacteria inoculation and N doses remained non-significant in this regard. Non-inoculated and inoculated seeds produced the longest (99.1 cm) and shortest (97.4 cm) plants, respectively. Similarly, N_3 (99.4 cm) and N_0 (96.9 cm) doses produced the longest and the shortest plants, respectively (Table 5). Being a leguminous plant, soybean depletes a significant quantity of N from the soil throughout its growth stages. The N application increased plant height in the current study. Bakal et al. [21] reported that the height of soybean plants ranged from 89.9 to 131.5 cm. Ilker [22] reported that plant heights of soybean ranged from 81.5 to 105.4 and 63.1 to 94.9 cm in a two-year study. Boydak et al. [23] studied yield-related traits of 12 different soybean varieties, and plant height values ranged from 79.37 to 126.07 cm in the first year and 67.90 to 102.80 cm during the second year. Sevilmiş [24] reported that soybean varieties planted on June 15 attained plant heights ranging from 75.2 to 111.5 cm. Ekinci [25] reported that plant height ranged from 37.27 to 98.67 cm in second-crop soybean. These studies are consistent with our findings.

Thousand Grain Weight (g)

Individual and interactive effects of bacteria inoculation and N doses significantly altered 1000-grain weight. Bacteria-inoculated and non-inoculated seeds resulted in the highest (218.1 g) and the lowest (211.3 g) 1000-grain weight, respectively. Similarly, the highest (217.1 g) and the lowest (207.4 g) 1000-grain weight was recorded for N_4 and N_0 doses, respectively. Regarding interaction, bacteria inoculation×N_3 doses resulted in the highest (220.3 g), whereas non-inoculated seeds×N_0 doses resulted in the lowest (198.4 g) 1000-grain weight (Table 6).

Seed size and composition are crucial characteristics of food crops and influenced by the availability of nutrients in the soil. Ohyama et al. [26] reported that seed weight directly influences yield along with seed count. The 1000-seed weight is a crucial and intricate characteristic in soybeans that has a significant role on crop productivity. Arıoğlu et al. [18] reported that 1000-grain weight ranged from 196.0 to 217.3 g under various bacteria inoculation methods in soybean. Seed inoculation with liquid bacteria before sowing resulted in the highest 1000-grain weight (217.3 g), whereas the control treatment had the lowest (196.0 g) 1000-grain weight. Özkul [20] reported that bacteria inoculation and N fertilization at various doses positively impacted 1000-seed weight in second-crop soybean cultivated in the Çukurova region. Application of N-fertilizer and seed inoculation with N-fixing bacteria such as *Bradyrhizobium* are essential for higher soybean yields. Several studies have demonstrated that seed inoculation with *B. japonicum* positively impacted soybean yield [27, 28].

Grain Yield (kg ha^{-1})

Grain yield was significantly ($p \leq 0.01$) influenced by individual effects of bacteria inoculation and N doses, whereas their interactive effect remained non-significant in this regard. The highest (3645 kg ha^{-1}) and the lowest (3072 kg ha^{-1}) grain yield was recorded for bacteria-inoculated and non-inoculated seeds, respectively. Similarly, N_7 (3628 kg ha^{-1}) and N_0 (2791 kg ha^{-1}) doses produced the highest and the lowest grain yield, respectively. Likewise, bacteria-inoculated seeds×N_7 dose resulted in the highest (3932 kg ha^{-1}) grain yield, whereas non-inoculated seeds×N_0 dose produced the lowest (2598 kg ha^{-1}) grain yield (Table 6). The present investigation revealed that application of N and seed inoculation with bacteria exhibited a favorable impact on the grain yield, leading to a significant increase in the yield. Liu et al. [29] discovered that seed yield is a multifaceted characteristic that is greatly affected by the interplay between genotype and environment. Bakal et al. [21] reported that the seed yield of 14 soybean varieties grown as a second crop in the Çukurova region ranged from 4667 to 3194 kg ha^{-1}. Gai et al. [30] found that application of 25, 50, and 75 kg N per hectare increased root activity, leaf photosynthesis, and yield of soybean. Arıoğlu et al. [18] found that liquid bacterial application resulted in the highest seed production (5056 kg ha^{-1}) in Çukurova, whereas non-inoculated treatment produced the lowest yield (3562 kg ha^{-1}). Stajković-Srbinović et al. [31] reported that seed inoculation with *Bradyrhizobium japonicum* and two *Pseudomonas* sp. affected soybean seed production and macronutrient absorption. They found that *B. japonicum* and *Pseudomonas* sp. improved soybean seed production from 65% to 134%. The results of the current study are consistent with earlier investigations.

Regression Analysis

Different N doses and seed inoccualtion with bacteria significantly altered soybean yield grown as a second crop. The correlation between N application and crop yield was established for fertilizer recommendations. The relationship between N doses and yield was determined using the average yield values (Table 4). The regression analysis resulted in a quadratic Equation expressed as $y = -0.1976x^2 + 8.9314x + 294.79$ for the relationship between N fertilizer and yield. According to this equation, the relationship between applied N and yield is depicted in Fig. 1.

The kilogram price of soybean and ammonium sulfate fertilizer used in the economic analysis was 11 and 7.50 TL, respectively. Furthermore, it was observed that effects of N doses on yield can be expressed by the Equation $Y = a + bx + cx^2$. Therefore, the parameters a, b, and c were calculated for the experimental site, and the relationship between N doses and yield was determined [20]. It is

Table 5. The impact of individual and interactive effects of bacteria inoculation and nitrogen doses on number of pods per plant and plant height of soybean.

Treatments	2021	2022	Mean	2021	2022	Mean
	Number of pods per plant			Plant height (cm)		
Bacteria inoculation (A)	**	**	**	*	**	**
Nitrogen doses (N)	**	**	**	**	**	**
A × N	**	**	**	ns	ns	ns
Years (Y)	-	-	**	-	-	**
Y × A	-	-	ns	-	-	ns
Y × N	-	-	ns	-	-	ns
Y × A × N	-	-	**	-	-	ns
Bacteria inoculation (A)						
Inoculated (A1)	81.7 a	92.4 a	87.1 a	93.0 b	101.8 b	97.4 b
Non-inoculated (A0)	77.0 b	88.1 b	82.6 b	94.8 a	103.3 a	99.1 a
LSD (0.05)	0.67	0.97	0.45	1.34	0.29	0.52
Nitrogen doses (N)						
N_0	78.2 cd	88.1 e	83.2 d	92.4 d	101.4 c	96.9 d
N_1	77.9 d	88.3 e	83.1 d	93.1 cd	101.8 bc	97.5 cd
N_2	79.2 b-d	89.9 d	84.6 c	93.8 bc	102.3 a-c	98.1 bc
N_3	80.1 ab	91.0 bc	85.6 ab	95.4 a	103.3 a	99.4 a
N_4	79.3 bc	90.2 cd	84.8 bc	95.2 a	103.4 a	99.3 a
N_5	81.0 a	91.7 ab	86.4 a	94.6 ab	102.9 a	98.8 ab
N_6	79.0 b-d	90.8 b-d	84.9 bc	93.7 bc	102.7 ab	98.2 bc
N_7	80.0 ab	92.2 a	86.1 a	93.1 cd	102.9 a	98.0 bc
LSD (0.05)	1.40	1.14	0.89	1.22	1.11	0.81
Interaction (A × N)						
$N_0 \times A_1$	82.4 ab	88.8 d-f	85.6 ef	90.9	99.9	95.4
$N_1 \times A_1$	80.4 c	89.2 d	84.8 f	92.4	100.2	96.3
$N_2 \times A_1$	80.2 c	92.3 c	86.3 de	93.0	101.2	97.2
$N_3 \times A_1$	83.7 a	94.1 ab	88.9 ab	95.1	103.2	99.2
$N_4 \times A_1$	81.5 bc	92.9 bc	87.2 cd	94.9	102.7	98.8
$N_5 \times A_1$	81.8 a-c	94.0 ab	87.9 bc	93.3	102.6	98.0
$N_6 \times A_1$	80.2 c	92.5 bc	86.4 de	92.7	102.5	97.6
$N_7 \times A_1$	83,4 ab	95.2 a	89.3 a	91.8	102.2	97.0
$N_0 \times A_0$	74.0 f	87.3 f	80.7 ı	94.0	102.8	98.4
$N_1 \times A_0$	75.4 ef	87.4 ef	81.4 hı	93.9	103.4	98.6
$N_2 \times A_0$	78.1 d	87.4 ef	82.8 g	94.6	103.4	99.0
$N_3 \times A_0$	76.5 de	87.9 d-f	82.2 gh	95.7	103.4	99.5
$N_4 \times A_0$	77.2 de	87.4 ef	82.3 gh	95.5	104.1	99.8
$N_5 \times A_0$	80.3 c	89.4 d	84.9 f	95.9	103.3	99.6
$N_6 \times A_0$	77.8 d	89.0 de	83.4 g	94.7	102.8	98.7
$N_7 \times A_0$	76.7 de	89.1 d	82.9 g	94.5	103.5	99.0
LSD (0.05)	1.97	1.62	1.25	ns	ns	ns
CV %	1.74	1.0	1.0	1.29	0.97	1.0

** = Significant at 0.01, * = significant at 0.05, ns = non-significant, The means sharing the similar letters are statistically non-significant, N_0 = Control, N_1= 40 kg ha^{-1} pure N, N_2 = 80 kg ha^{-1} pure N, N_3 = 120 kg ha^{-1} pure N, N_4 = 160 kg ha^{-1} pure N, N_5 = 200 kg ha^{-1} pure N, N_6 = 240 kg ha^{-1} pure N, N_7 = 280 kg ha^{-1} pure N.

Table 6. The influence of individual and interactive effects of bacteria inoculation and nitrogen doses on thousand grain weight and grain yield of soybean.

Treatments	2021	2022	Mean	2021	2022	Mean
	Thousand grain weight (g)			Grain yield (kg ha^{-1})		
Bacteria inoculation (A)	**	**	**	**	**	**
Nitrogen doses (N)	**	**	**	**	**	**
A × N	**	**	**	ns	ns	ns
Years (Y)	-	-	ns	-	-	*
Y × A	-	-	ns	-	-	ns
Y × N	-	-	ns	-	-	ns
Y × A × N	-	-	ns	-	-	ns
Bacteria inoculation (A)						
Inoculated (A1)	218.0 a	218.2 a	218.1 a	3522 a	3768 a	3645 a
Non-inoculated (A0)	211.3 b	211.2 b	211.3 b	2981 b	3162 b	3072 b
LSD (0.05)	2.05	1.16	0.90	23.96	32.54	15.53
Nitrogen doses (N)						
N_0	207.2 d	207.6 b	207.4 e	2671 c	2909 c	2791 c
N_1	215.0 a-c	214.8 a	214.9 cd	2777 c	3088 c	2933 c
N_2	217.5 a	215.1 a	216.3 a-c	3067 b	3334 b	3201 b
N_3	216.5 a-c	216.6 a	216.6 ab	3464 a	3587 a	3526 a
N_4	217.1 ab	217.0 a	217.1 a	3498 a	3654 a	3576 a
N_5	214.1 c	214.9 a	214.5 d	3504 a	3699 a	3602 a
N_6	214.9 a-c	215.8 a	215.3 b-d	3509 a	3713 a	3611 a
N_7	214.8 bc	216.0 a	215.4 a-d	3524 a	3733 a	3628 a
LSD (0.05)	2.65	2.22	1.70	21.99	23.06	15.70
Interaction (A × N)						
$N_0 \times A_1$	216.9 a-d	216.0 b-d	216.5 d-f	2842	3125	2983
$N_1 \times A_1$	219.9 ab	219.6 a	219.8 ab	3014	3365	3189
$N_2 \times A_1$	217.9 a-c	217.7 a-c	217.8 b-e	3339	3710	3524
$N_3 \times A_1$	220.2 a	220.4 a	220.3 a	3794	3917	3856
$N_4 \times A_1$	219.2 ab	219.1 ab	219.2 a-c	3760	3958	3859
$N_5 \times A_1$	215.4 c-e	215.9 cd	215.6 e-g	3787	4002	3894
$N_6 \times A_1$	216.2 b-e	218.0 a-c	217.1 c-f	3819	4024	3922
$N_7 \times A_1$	218.4 a-c	219,2 a	218.8 a-d	3824	4039	3932
$N_0 \times A_0$	197.5 g	199.2 g	198.4 k	2501	2695	2598
$N_1 \times A_0$	210.0 f	210.0 f	210.0 j	2540	2812	2676
$N_2 \times A_0$	217.0 a-d	212.4 ef	214.7 f-h	2795	2959	2877
$N_3 \times A_0$	212.9 ef	212.9 d-f	212.9 hı	3135	3256	3196
$N_4 \times A_0$	215.1 c-e	214.9 c-e	215.0 f-h	3235	3350	3293
$N_5 \times A_0$	212.9 ef	213.9 de	213.4 g-ı	3222	3397	3309
$N_6 \times A_0$	213.5 d-f	213.6 de	213.6 g-ı	3199	3401	3299
$N_7 \times A_0$	211.3 f	212.9 d-f	212.1 ıj	3223	3427	3325
LSD (0.05)	3.74	3.14	2.40	ns	ns	ns
CV %	1.22	1.00	1.00	6.70	6.00	6.00

** = Significant at 0.01, * = significant at 0.05, ns = non-significant, The means sharing the similar letters are statistically non-significant, N_0 = Control, N_1 = 40 kg ha^{-1} pure N, N_2 = 80 kg ha^{-1} pure N, N_3 = 120 kg ha^{-1} pure N, N_4 = 160 kg ha^{-1} pure N, N_5 = 200 kg ha^{-1} pure N, N_6 = 240 kg ha^{-1} pure N, N_7 = 280 kg ha^{-1} pure N.

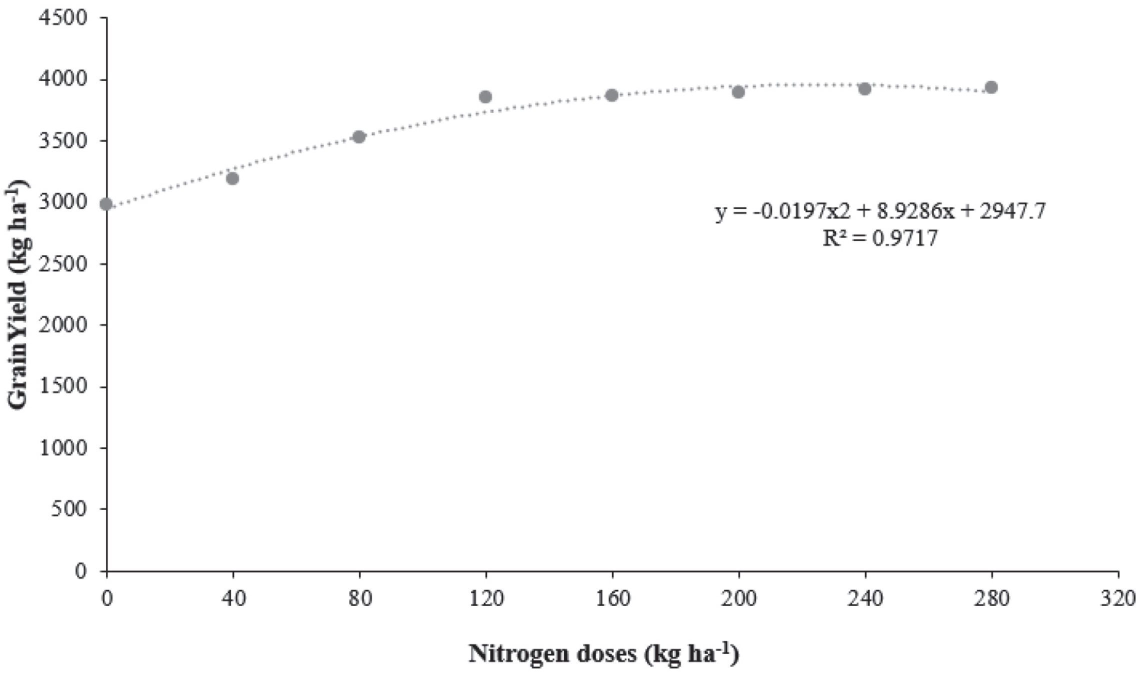

Fig. 1. The relationship between nitrogen doses and grain yield of bacteria-inoculated soybean sown as second crop.

necessary to calculate the cost of 1 kg of pure N fertilizer. Ammonium sulfate fertilizer contains 21% pure N; therefore, the price of 1 kg of pure N was computed as:

$$Fg = [(7.5 \times 100) \times 1] / 21.0 \quad (1)$$
$$Fg = 35.71 \text{ TL}$$

The economic fertilizer dose was calculated by using the Equation given below:

$$Eg = (Fg\text{-}Fm \times b) / (2 \times Fm.c) \quad (2)$$

Here, Fg = unit price of fertilizer, Fm = unit price of produce, b = linear effect of fertilizer (bx), and c = quadratic effect of fertilizer (cx^2).

$$Eg = (35.71\text{- } (11 \times 8.9314)) / (2 \times 11.0 \times\text{-}0.1976)$$
$$Eg = 144 \text{ kg ha}^{-1} \text{ pure N}$$

According to this, the optimum economic pure N required for soybean cultivation under the climatic conditions of Şanlıurfa was 144 kg ha^{-1}.

Protein Ratio (%)

Individual and interactive effects of bacteria inoculation and N doses significantly altered protein ratio. The highest (39.6%) and the lowest (38.9%) protein ratio was noted from bacteria-inoculated and non-inoculated seeds, respectively. Similarly, N_7 (39.8%) and N_0 (%38.7) doses resulted in the highest and the lowest protein ratio. Regarding the interactive effect, bacteria-inoculated seeds×N_7 resulted in the highest (40.1%), while non-inoculated seeds×N_0 resulted in the lowest (37.9%) protein ratio (Table 7). The current study found that bacteria inoculation and nitrogen application improved protein ratio. Bakal et al. [21] reported that the protein content ranged from 36.52% to 38.46% in the Çukurova region.

Oil Ratio (%)

The combined two-year data analysis indicated that oil ratio was significantly ($p \leq 0.01$) influenced by individual effects of bacteria inoculation and N doses. However, the interactive effect of bacteria inoculation and N doses remained non-significant in this regard. The highest (18.4%) and the lowest (17.6%) oil ratio was recorded for non-inoculated and bacteria-inoculated seeds, respectively. Similarly, N_1 and N_6 and N_7 resulted in the highest (18.4%) and the lowest (17.6%) oil ratio (Table 7). Fabre and Planchon [32] reported a strong correlation between oil and protein ratio and nitrogen fertilization in soybean. Specifically, the efficacy of root nodules formed during the growth phase and the availability of usable nitrogen in the soil are significant factors impacting the oil and protein ratio. It has been observed that an increase in available nitrogen decreases the oil ratio and increases the protein ratio.

Conclusions

Soybeans require a high amount of N; however, fulfilling this demand through fertilization is not economically feasible. Soybean plants obtain a portion of their required N from the soil in a readily available form, while a significant portion is met through the aid of *Rhizobium* bacteria

Table 7. The influence of individual and interactive effects of bacteria inoculation and nitrogen doses on protein and oil ratio of soybean.

Treatments	2021	2022	Mean	2021	2022	Mean
	Protein ratio (%)			Oil ratio (%)		
Bacteria inoculation (A)	**	**	**	*	**	**
Nitrogen doses (N)	**	**	**	**	**	**
A × N	**	ns	**	**	ns	ns
Years (Y)	-	-	**	-	-	**
Y × A	-	-	ns	-	-	**
Y × N	-	-	ns	-	-	**
Y × A × N	-	-	**	-	-	ns
Bacteria inoculation (A)						
Inoculated (A1)	40.3 a	38.9 a	39.6 a	16.4 b	18.8 b	17.6 b
Non-inoculated (A0)	39.7 b	38.0 b	38.9 b	16.6 a	20.1 a	18.4 a
LSD (0.05)	0.13	0.44	0.17	0.10	0.37	0.14
Nitrogen doses (N)						
N_0	39.6 b	37.8 e	38.7 e	16.5 bc	20.2 a	18.3 ab
N_1	39.7 b	38.0 de	38.8 de	16.6 a	20.2 a	18.4 a
N_2	39.7 b	38.3 cd	39.0 d	16.6 ab	19.9 ab	18.3 ab
N_3	40.1 a	38.5 bc	39.3 c	16.7 a	19.7 a-c	18.2 ab
N_4	40.2 a	38.6 bc	39.4 bc	16.6 a	19.4 b-d	18.0 bc
N_5	40.1 a	38.6 bc	39.4 bc	16.3 d	19.2 c-e	17.8 cd
N_6	40.3 a	38.9 ab	39.6 ab	16.4 cd	18.8 de	17.6 d
N_7	40.3 a	39.2 a	39.8 a	16.4 cd	18.7 e	17.6 d
LSD (0.05)	0.27	0.43	0.25	0.12	0.65	0.32
Interaction (A × N)						
$N_0 \times A_1$	40.4 a	38.5	39.4 c-e	16.3 ef	19.2	17.7
$N_1 \times A_1$	40.3 ab	38.3	39.3 d-f	16.5 c-e	19.5	18.0
$N_2 \times A_1$	40.3 ab	38.4	39.3 d-f	16.6 a-d	19.1	17.8
$N_3 \times A_1$	40.5 a	38.7	39.6 b-d	16.8 a	19.1	17.9
$N_4 \times A_1$	40.5 a	39.1	39.8 a-c	16.7 a-c	18.5	17.6
$N_5 \times A_1$	40.2 ab	39.0	39.6 b-d	16.2 f	18.6	17.4
$N_6 \times A_1$	40.3 ab	39.5	39.9 ab	16.3 ef	18.3	17.3
$N_7 \times A_1$	40.2 ab	39.9	40.1 a	16.2 f	18.4	17.3
$N_0 \times A_0$	38.8 e	37.0	37.9 j	16.7 ab	21.1	18.9
$N_1 \times A_0$	39.1 de	37.6	38.3 ı	16.8 a	20.8	18.8
$N_2 \times A_0$	39.2 d	38.1	38.6 hı	16.6 a-d	20.8	18.7
$N_3 \times A_0$	39.7 c	38.2	38.9 gh	16.6 a-d	20.2	18.4
$N_4 \times A_0$	40.0 bc	38.1	39.0 fg	16.6 a-d	20.3	18.4
$N_5 \times A_0$	40.0 bc	38.2	39.1 e-g	16.5 b-d	19.7	18.1
$N_6 \times A_0$	40.3 ab	38.2	39.2 d-g	16.5 de	19.3	17.9
$N_7 \times A_0$	40,5 a	38.6	39.5 b-d	16.6 a-d	19.0	17.8
LSD (0.05)	0.38	ns	0.35	0.18	ns	ns
CV %	0.67	1.00	1.00	0.75	3.00	2.00

** = Significant at 0.01, * = significant at 0.05, ns = non-significant, The means sharing the similar letters are statistically non-significant, N_0 = Control, N_1 = 40 kg ha^{-1} pure N, N_2 = 80 kg ha^{-1} pure N, N_3 = 120 kg ha^{-1} pure N, N_4 = 160 kg ha^{-1} pure N, N_5 = 200 kg ha^{-1} pure N, N_6 = 240 kg ha^{-1} pure N, N_7 = 280 kg ha^{-1} pure N.

residing in their roots, which fix atmospheric N. This study investigated the effects of bacteria inoculation and different N doses on yield and certain quality parameters of soybean grown as a second crop in Şanlıurfa Province, Türkiye. The results revealed that bacteria inoculation and different N doses significantly altered the yield and quality traits of soybean. The grain yield of the plants receiving no N and no bacteria inoculation was significantly lower than the plants fertilized with N and seeds inoculated with bacteria. The results revealed that bacteria inoculation and N application exerted positive impacts on yield and quality of soybean. The N_7 dose along with bacteria inoculation improved grain yield by 51.35% compared to non-inoculated seeds receiving no N. The highest grain yield (3932 kg ha^{-1}) was noted for bacteria-inoculated seeds × N_7 interaction, whereas non-inoculated seeds × N_0 interaction produced the lowest grain yield (2598 kg ha^{-1}). The results further revealed that grain yield linearly increased till the application of 144 kg ha^{-1} pure N; however, the yield increase beyond this N amount was minimal. Therefore, it is not recommended to apply N fertilizers > 144 kg ha^{-1} due to the cost outweighing the resulting seed yield. In conclusion, the optimal economic pure N amount for the cultivation of the 'Gapsoy 16' soybean variety in Şanlıurfa is 144 kg ha^{-1}.

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Conflict of Interest

The authors declare no conflict of interest.

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