Turkish Journal of FIELD CROPS

Turk J Field Crops
2023, 28(1), 15-25
DOI: 10.17557/tjfc.1189103

INTERACTIVE EFFECTS OF N FERTILIZATION AND *BRADIRHIZOBIA JAPANICUM* ON AGRONOMICAL TRAITS OF SOYBEAN IN SALT AFFECTED SOILS

Inomjon ISRAILOV[1], Khamidulla SHERALIEV[1], Guliston ABDALOVA[1], Abduvali IMINOV[1], Kholik ALLANOV[1], Aziz KARIMOV[2], Botir KHAITOV[2], Young Chang KIM[3*]*

[1]Tashkent State Agrarian University, Faculty of Agrobiology, str. University 1, Tashkent 100140, Uzbekistan
[2]International Center for Biosaline Agriculture, Regional office for Central Asia and the South Caucasus, Tashkent 100084, Uzbekistan
[3]Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science, Rural Development Administration, Eumseong, Chungbuk 27709, Republic of Korea
[*]Corresponding authors: b.khaitov@biosaline.org.ae, ycpiano@korea.kr

Received: 14.10.2022

ABSTRACT

Soil salinity has enormous negative impact on crop productivity leading to food insecurity and malnutrition, especially in arid regions. The aim of this study was to elucidate the interactive effects of different N fertilization rates and *Bradyrhizobium japonicum* (Kirchner) on agronomic traits of soybean [*Glycine max* (L.) Merr.] in saline soils (EC 5.8 dS m^{-1}). Field experiments were conducted during the summer seasons of 2018 and 2019 to evaluate the effects of various N fertilization rates such as no fertilization (control), $N_0P_{90}K_{60}$, $N_{30}P_{90}K_{60}$, $N_{60}P_{90}K_{60}$ individually and in tandem with *B. japonicum* as a seed bioinoculant. The experiment in a split-plot design, N fertilization as the main plot, the seed inoculation as the sub-plot was set up in three replicates. Soybean growth, nutrients uptake and yield parameters increased with increasing N fertilization rate, however, the effect was more pronounced with the seed inoculation. Averaged over the cropping seasons, yield was higher by 20.4%, 19.0%, 34.1% and 6.1% in the inoculated treatments of no-fertilization, $N_0P_{90}K_{60}$, $N_{30}P_{90}K_{60}$, $N_{60}P_{90}K_{60}$, respectively as compared to the similar fertilization treatments without the seed inoculation. The fertilization rate of $N_{30}P_{90}K_{60}$ with *B. japonicum* inoculation was recommended due to the high soybean yield and quality seeds as the crucial components of sustainable agricultural production under salt-stressed field conditions.

Keywords: *Bradyrhizobium japonicum*, *Glycine max,* N fertilization, salinity, plant growth, nutrient content, soybean, yield.

INTRODUCTION

Soybean, *Glycine max* (L.) Merr., is one of the widely produced legume species, mainly for oil production around the globe (FAOSTAT, 2020). In fact, soybean oil constitutes approximately 29% of the global vegetable oil production (SOYSTATS, 2020). Soybean grain consists of a valuable source of plant proteins (36%) with essential fatty acid (FA) composition. Intrinsically, soybean oil contains zero cholesterol and saturated fatty acids such as palmitic (16:0) and stearic (18:0) acids and higher concentrations of unsaturated FA such as oleic (18:1), linoleic (18:2), and linolenic (18:3) acids and some bioactive compounds such as phenols, flavonoids, and organic acids make it valuable for the human diet (Ilker et al., 2018a; Szpunar-Krok et al., 2021).

It is well studied that legumes such as soybeans, peas (*Pisum sativum* L.), chickpeas (*Cicer arietinum* L.), peanuts (*Arachis hypogaea* L.) and etc. with beneficial microorganisms such as rhizobia and bradyrhizobia bacteria may potentially reduce the application of chemical fertilizers through the function of biological nitrogen fixation (BNF) (Htwe et al., 2019; Kocaturk et al., 2019; Nosheen et al., 2021). Indeed, the expanded interest in sustainable green development and ecology has drawn attention to the fact that BNF is an ecologically friendly and renewable resource (Mahanta et al., 2014; Jaga and Sharma, 2015; Ilker et al., 2018b). Nowadays, many organic farmers have been using legumes with appropriate bacterial symbiosis in crop rotation systems to improve soil fertility and crop productivity (Silva et al., 2017; Wang et al., 2018; Allanov et al., 2019). It is noteworthy that this practice provides additional economic and ecological benefits (Son et al., 2004). Cultivation agrotechnologies, environmental conditions and soil properties play a vital role in the symbiotic association between soybean and

Bradyrhizobium and their proper functioning (Abdelghany et al., 2020). As soybean in association with *Bradyrhizobium* bacteria may improve soil health can be helpful for subsequent crops (Azam et al., 2021), however abiotic and biotic stress factors may adversely affect soybean productivity.

The amount of fixed nitrogen from air by the soybean and rhizobia co-existence ranges between 0 to 337 kg per ha depending on external factors (Zimmer et al., 2016). Interestingly, the N fixation process made by the legume association decreases when the soil has abundant N resources and vice versa (Marro et al., 2020). Numerous studies indicated that soybean needs small amounts of N fertilization at the early growth stage to overcome nitrogen deficiency (Sulieman et al., 2015; Cafaro La Menza et al., 2019). At this period, the absence of nitrogen fertilizer may negatively affect soybean development and yield characteristics. In contrast, excessive fertilization negatively affects plant growth, soil health, and crop economic yield (da Mota et al., 2019; Ozturk, 2021).

Despite adverse impacts of soil salinity on crop production, new biotechnological and agrotechnological approaches have added wings to the progress of plant studies with the potential to alleviate stress and promote growth under saline aquifer (Toker et al., 2009; Stojsin et al., 2022). Soybean with *B. japonicum* (Kirchner) symbiosis has been intensively studied under various environments, however, the effectiveness of this symbiosis with different N fertilization under saline conditions has not been worked out. Therefore, it becomes an immense need to study the effect of various N fertilization norms in conjunction with *Bradyrhizobium* inoculation on soil properties, soybean growth, nutrient uptake, seed yield and seed chemical compositions. The main objective of this study was to reveal the effectiveness of the seed inoculation using *B. japonicum* in association with the appropriate rates of N fertilization on enhancing soybean productivity and soil quality under saline environment.

MATERIALS AND METHODS

Experiment area and design

The field experiments were conducted during two successive summer growing seasons of 2018 and 2019 at the experimental station of the Cotton Breeding, Seed Production and Agrotechnologies Research Institute located in Guliston district, Syrdarya region, Uzbekistan (Altitude: 271 m, Latitude: 40°25′ North, Longitude: 68°40′ East). In this area, the continental arid climate is predominant with four distinctive seasons. The crop growing season is characterized as hot and mostly dry with frequently drought accompanying with heat stress. The higher air temperature was observed in July up to 38^0C and lower temperature in January 0^0C. The frost-free period is 240-270 days. The mean annual rainfall ranges between 210 and 245 mm. Cultivated crops do not generally benefit from it because a major part falls in the period between December and March (Fig.1).

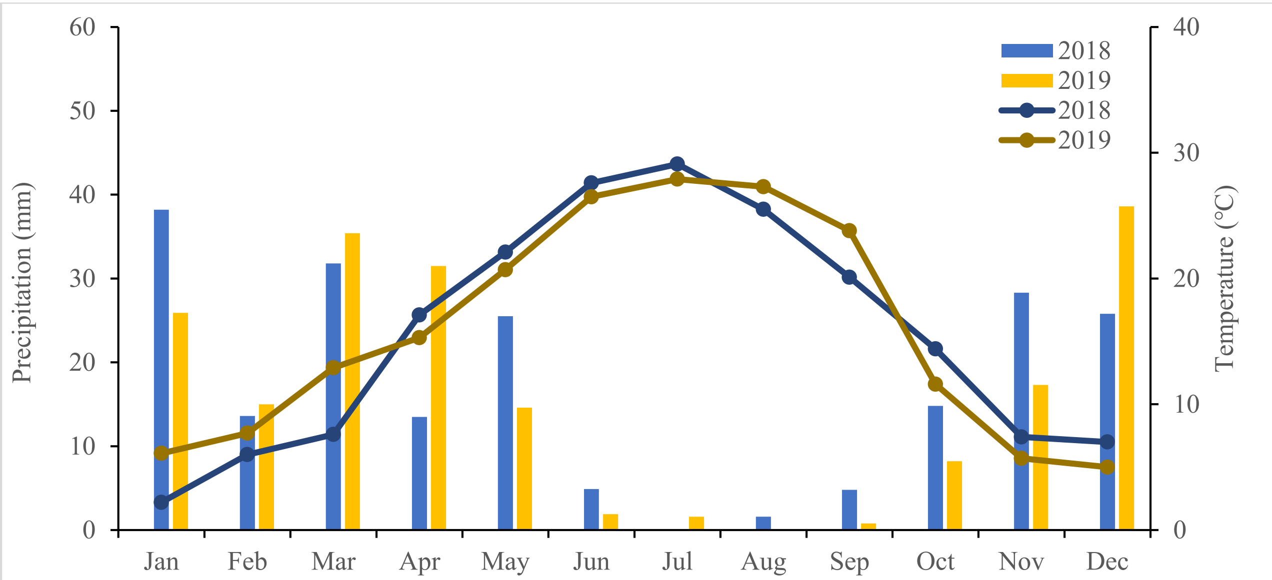

Figure 1. Weather conditions during soya bean growing season in the years 2018-2019.

The experiment was set up in randomized complete block design arranged in split-plots with three replications which consists of 24 plots (each plot size 4.8 m × 10 m = 48 m^2) and the total experiment area was 1152 m^2. The selected soybean genotype has been subjected to grow under unfertilized (control) and three N fertilization treatments with and without inoculation with *Bradyrhizobium japonicum* (Kirchner). The recommendations for the bacterial inoculation process made by the manufacturer were strictly followed with the protocol. The N fertilization rates were: 0, 30, 60 kg·per ha and each plot received P 90 kg·per ha and K 60 kg·per ha except the control treatment. The experiment design consists of eight fertilization treatments with and without *B. japonicum* inoculation: no fertilization (control), $N_0P_{90}K_{60}$, $N_{30}P_{90}K_{60}$, $N_{60}P_{90}K_{60}$. Crop cultivation technologies including irrigation, weed control and pest management were conducted in accordance with local agronomic practices, while maintaining similarity for all trials. Chemical fertilizers were applied during the growing season as a band placement. Nitrogen (N) at a rate of 30 kg per ha was applied at sowing based on the treatment features. Phosphorous (P) and Potassium (K) fertilizers in the form of triple superphosphate (19.8% P) and muriate of potash (21.5% K) were split into two equal portions and applied with the remained portion of Nitrogen (N) prior to the first and the second irrigations to the appropriate experiment treatments. Four times surface furrow irrigation was applied equally to all plots with a norm of 750-800 m^3 per hectare during the vegetation period.

Bacterial Strain and Soybean Seed

Strain *B. japonicum* was kindly provided from Microbiology Institute, Uzbek Academy of Science, Tashkent, Uzbekistan. The strain was ready for inoculation after having the cell concentration adjusted to 3×10^9 CFU per ml. Following the bacterial cultivation, the seeds were soaked entirely with the N_2 fixing bacteria and kept in the shade for half an hour in to dry and be ready for planting.

The soybean genotype Orzu was used in this experiment which is characterized as early ripening. Before planting, the surface of seeds were sterilized with 70% ethanol for 1 min and 3 g per L sodium hypochlorite solution for 3 min. These procedures were followed with washing of the seeds under tap water. After two hours of drying in the lab condition, the seeds were completely soaked with the bacterial strain for 15 minutes. The inoculated seeds of this crop species were planted in 3-4 cm deep with the planned norms.

Soil characteristics and sample analyses

The soil in this area is typical sierozem affected by secondary salinization (EC 5.8 dS m^{-1}) after many years of improper irrigation used for cultivated crops. The mechanic structure is heavy sandy without sufficient organic matters and nutrients the underground water level is deep (2-3 m). Poor soil structure and low organic matters are prevalent in this area with 0.7-0.9% humus content and 1.3-1.4 g/cm^3 bulk density. The pH of the soil was neutral (6.8-6.9), the total organic carbon was 0.09-0.87%. The average nutrient contents in the 0–30 and 30–50 cm soil horizon were 0.08 and 0.07% total N and 0.168 and 0.154% total P, whereas exchangeable potassium levels ranged between 266 and 207 mg per kg, respectively (Table 1).

Table 1. Nutrient content in soil as affected by *B. japonicum* inoculation and N fertilization (averaged across 2018 and 2019 growth seasons).

Treatments	Soil horizon (cm)	Total forms (%)			Exchangeable forms (mg per kg)		
		Humus	N	P	NO_3	P_2O_5	K_2O
At the beginning of the experiment							
	0-30	0.871b	0.078b	0.168c	1.23f	24.24b	266c
	30-50	0.733d	0.066c	0.154e	0.73g	19.28d	207d
At the vegetation period							
Control	0-30	0.872b	0.077 b	0.165c	3.24d	23.94b	262c
	30-50	0.735d	0.064c	0.155e	2.12e	18.48e	205d
Control+Br	0-30	0.895a	0.079b	0.168c	3.11d	25.26b	276b
	30-50	0.751c	0.065c	0.157d	2.69e	19.51d	207d
$N_0P_{90}K_{60}$	0-30	0.897a	0.080b	0.170b	4.15c	24.78b	276b
	30-50	0.754c	0.066c	0.158d	2.98e	20.10c	209d
$N_0P_{90}K_{60}$+Br	0-30	0.876b	0.080b	0.171b	4.36c	27.15a	286ab
	30-50	0.753c	0.065c	0.159d	3.43d	20.17c	217d
$N_{30}P_{90}K_{60}$	0-30	0.902a	0.086a	0.173a	5.54ab	25.99b	284ab
	30-50	0.759c	0.066c	0.159d	3.08d	20.63c	217d
$N_{30}P_{90}K_{60}$+Br	0-30	0.905a	0.087a	0.174a	5.92a	27.98a	296a
	30-50	0.760c	0.067c	0.158	3.65d	20.90c	217d
$N_{60}P_{90}K_{60}$	0-30	0.898a	0.084a	0.171b	5.23b	26.55ab	280ab
	30-50	0.755c	0.066c	0.158d	3.43d	20.78c	214d
$N_{60}P_{90}K_{60}$+Br	0-30	0.902a	0.086a	0.172b	5.74a	27.24a	286ab
	30-50	0.758c	0.065c	0.159d	3.32d	20.62c	209d

Means of three independent replications (n = 3) separated by lower case letters (a and e) in each column are significantly different at $P \leq 5\%$.

There was no previous legume cultivation with rhizobia in this experimental site. The preceding crop was winter wheat which was harvested in the middle of June. As a second crop, soybean was planted in late June and harvested in early October during both 2018–2019 cropping seasons. Several microbiological analyses were conducted to determine the microbial activity of soil samples at the beginning and end of the experiment. The Serial dilution plate method was employed to observe total numbers of actinomycetes, ammonifiers and oligonitrophils by counting colony-forming units (CFUs) on agar plates. The medium used for enumeration of total numbers of spores and micromycetes was soil agar.

The roots and shoots samples were oven-dried at 72^0C for 72 hours. These materials were ground and sieved before chemical analysis. The Kjeldahl method was applied to determine the total nitrogen content in the roots and shoots samples using with a nitrogen analyzer (Kjeltec 2300 Autoanalyser, Foss Tecator AB, Hoganas, Sweden). The Tyurin and Lancaster methods were useful for analyses of soil organic matters and available phosphate (NIAST, 2000). In addition, the flame photometer model carl-Zeiss was implemented to study phosphorus as described by Horneck and Hanson (1998). Potassium content was analyzed by the inductively coupled plasma spectrophotometer.

Total oil content was recorded in the Soxhlet extractor according to official methods (Januszewska et al., 1999). While seed protein was estimated by the neutron activation analysis method to determine seed protein content. Fatty acid composition of soybean seed oil was determined with derivatization fatty acid (FA) as methyl ester (FA-ME) used by directing transmethylation of seed oil with KOH methanol (Wirasnita et al., 2013).

Statistical Analyses

The obtained data consisting of soil nutrients, microflora activity, plant growth, chemical content, yield parameters were subjected to analysis of variance (ANOVA) using CropSTAT software. The Tukey test was employed to calculate for comparison of traits at $P < 0.05$.

RESULTS AND DISCUSSION

Soil nutrient content

The application of N fertilization and *B. japonicum* for soybean cultivation increased humus, total N and P content in the soil, while this significantly was different from the control treatment (Table 1). Also, the exchangeable NO_3, P_2O_5 and K_2O contents in the soil increased with increasing N rate. In addition to these positive effects, the effect was more pronounced with the bacterial inoculation. Nevertheless, exchangeable forms of NO_3, P_2O_5 and K_2O contents reached a maximum when the seed inoculant interacted with $N_{30}P_{90}K_{60}$ fertilization treatment. Particularly, soil chemical properties, i.e., exchangeable forms of NO_3 increased by four-fold, whereas P_2O_5 and K_2O rates were higher by 15.4 and 8.4% and 11.3 and 4.8% at 0-30 and 30-50 cm soil horizons, respectively compared to the control. Further increase of N fertilization rate up to 60 kg ha^{-1} with *B. japonicum* inoculation in the $N_{60}P_{90}K_{60}$×*B. japonicum* treatment did not show any additional effect (Table 1).

A similar trend was observed by the side of soil microbial activities, the seed inoculant along with $N_{30}P_{90}K_{60}$ fertilization treatment increased soil microbial activity, i.e., ammonifiers, spores, oligonitrophils, micromycetes, actinomycete (Table 2). The microbial biostimulant combined with the $N_{30}P_{90}K_{60}$ fertilization rate resulted in the highest number of the above-mentioned soil bacterial populations. An application of N combined with *B. japonicum* inoculation significantly affected nutrient availability in soil, plant physiological processes, and nutrient activities in the plant tissue (Table 2). The beneficial association between soybean and *B. japonicum* is mostly reflected in the content of N in the soil. The fixed amount of N ranges depending on the suitable match of the two organisms, external and anthropogenic factors (Silva et al., 2017). When N fertilizer combined with *B. japonicum*, remarkable improvement was revealed in terms of soil humus content. Likewise, the highest contents of total N and P were found when the $N_{30}P_{90}K_{60}$ fertilization treatment was used in combination with *B. japonicum*. Enhanced nutrient availability maintains soil ameliorative functions and rejuvenates of soil ecology. In contrast, the excessive use of N fertilizer negatively affected soil structure and fertility, suggesting that the disproportionate N application suppresses nutrient cycling, soil functioning, and health. Indeed, the highest productivity of crops largely depends on the rational implementation of chemical fertilizers even without damage to soil fertility under adverse climatic conditions (Ashraf and Harris, 2013). The N cycle in the soil had significant positive correlations with the $N_{30}P_{90}K_{60}$ fertilization rate in the association of *B. japonicum* inoculation. In its turn, increasing the total form of N in the soil is strongly coupled with exchangeable NO_3, P_2O_5 and K_2O contents. A similar phenomenon was promoted in early studies, confirming these macronutrients are stoichiometrically linked together to maintain ecosystem balancing (Aziz et al., 2013). This study showed that the use of the bacterial inoculant together with the appropriate N fertilization enhanced soil microbial diversity and population in saline soils. In fact, soil available nutrient content mostly depends on an abundance of soil microorganisms which play a fundamental role in the soil nutrient cycling and soil ecosystem functioning (Khan et al., 2010; Hammerschmiedt et al., 2021). The highest microbial activity was found when the seed inoculation was used with $N_{30}P_{90}K_{60}$ fertilization, suggesting that the application of N fertilizer at the right doses increases the biological quality of soil. Perhaps, further increase N rate ($N_{60}P_{90}K_{60}$) showed the harmful influence on the microbial community. This practice was well-documented previously, presenting symbiotic N_2 fixation in legumes with effective rhizobia play an essential role in compensating for missing soil nitrogen (N) and thus potentially surmount nutrient deficiency (Abdiev et al., 2019; Asik and Arioglu, 2020). Taken together, rejuvenation of beneficial soil bacteria had an enormous advantage for improving soil biological functions and plant health.

Table 2. Quantity of microorganisms in the rhizosphere as affected by *B. japonicum* inoculation and N fertilization (averaged across 2018 and 2019 growth seasons).

Treatments	Ammonifiers	Oligonitrophils	Micromycetes	Actinomycete
At the beginning of the experiment				
	$5.2x10^7$d	$3.1x10^6$c	$5.5x10^4$c	$4.2x10^5$b
At the vegetation period				
Control	$7.5x10^6$c	$4.5x10^5$d	$3.5x10^4$d	$7.5x10^4$e
Control+Br	$7.5x10^7$b	$2.7x10^6$c	$6.7x10^4$b	$3.5x10^5$c
$N_0P_{90}K_{60}$	$7.5x10^6$c	$5.2x10^5$d	$4.5x10^4$d	$1.5x10^5$d
$N_0P_{90}K_{60}$+Br	$7.5x10^7$b	$3.5x10^6$b	$7.5x10^4$b	$4.5x10^5$b
$N_{30}P_{90}K_{60}$	$7.5x10^7$b	$3.7x10^6$b	$6.5x10^4$b	$2.2x10^5$c
$N_{30}P_{90}K_{60}$+Br	$7.5x10^8$a	$4.5x10^6$a	$8.5x10^4$a	$6.5x10^5$a
$N_{60}P_{90}K_{60}$	$6.0x10^7$bc	$9.7x10^5$d	$5.7x10^4$c	$2.5x10^5$c
$N_{60}P_{90}K_{60}$+Br	$9.0x10^7$b	$3.7x10^6$b	$7.5x10^4$b	$5.5x10^5$ab

Means of three replications (n = 3) separated by lower case letters (a and e) in each column are significantly different at $P \leq 5\%$.

Soybean growth and plant nutrient uptake

The positive interaction between N fertilization rate with *Bradirhizobia* inoculation was observed in the dry weight parameters of soybean, whereas the combination of $N_{30}P_{90}K_{60}\times$ *B. japonicum* showed the highest values in all studied plant growth indicators (Table 3). The dry weights of root, shoot, leaf, grain and grain husk were increased with the increase of N fertilization rate. However, the increase of these parameters was more pronounced with *B. japonicum* inoculation. The root, shoot, leaf and grain dry weights increased by 20.4, 93.1, 87.5 and 107.5% when $N_{30}P_{90}K_{60}$ fertilization was used in combination with *B. japonicum* inoculation, while the total weight was higher by 78.0% as compared to the control. However, further increase of fertilization rate up to $N_{60}P_{90}K_{60}$ with and without *B. japonicum* did not exhibit any positive effect, on the contrary there were significant declines at the studied parameters compared to the $N_{30}P_{90}K_{60}$ fertilization treatment.

Table 3. Dry weight of the plant organs in response to N fertilization and *B. japonicum* inoculation (averaged across 2018 and 2019 growth seasons).

Treatments	Root (g)	Shoot, (g)	Leaf (g)	Grain (g)	Husk (g)	Total (g)
Control	4.9d	5.8e	0.8d	4.0e	2.7e	18.2e
Control+Br	5.2c	6.2e	1.0c	5.1d	3.5d	21.0d
$N_0P_{90}K_{60}$	5.2c	7.6d	1.1c	6.2c	4.2c	24.4c
$N_0P_{90}K_{60}$+Br	5.3c	7.1d	1.1c	7.1bc	4.8bc	25.4c
$N_{30}P_{90}K_{60}$	5.5b	9.3c	1.3b	7.9ab	5.4ab	29.4b
$N_{30}P_{90}K_{60}$+Br	5.9a	11.2a	1.5a	8.3a	5.6a	32.4a
$N_{60}P_{90}K_{60}$	5.8a	10.8b	1.4a	7.4b	5.1b	30.5ab
$N_{60}P_{90}K_{60}$+Br	5.6b	9.7c	1.3b	8.0ab	5.4ab	30.0ab

Means of three replications (n = 3) separated by lower case letters (a and e) in each column are significantly different at $P \leq 5\%$.

The application of chemical fertilization increased N, P and K contents in the root, shoot, leaf, grain and grain husk of soybean with significant differences (Tables 4, 5, and 6). Nevertheless, these indicators were significantly higher in the *B. japonicum* inoculated treatments, indicating *B. japonicum* inoculation promotes nutrient uptake. The content of these macro-elements in the soybean organs increased with the increase of N fertilizer application rate. The highest N, P and K elements values in the soybean organs was found at the $N_{30}P_{90}K_{60}$ fertilization treatment in combination with the seed inoculant. The N content in the soybean parts grown with *B. japonicum* inoculation increased by 30.7%, 33.3%, 8.1%, 28.5%, and 49.0% in the root, shoot, leaf, grain and grain husk, respectively compared to the control values. However, a decrease of these studied parameters was observed when $N_{60}P_{90}K_{60}$ fertilization treatment was cooperated with the seed inoculation.

A similar trend was also detected when P and K contents in soybean organs were studied in response to increasing N fertilization associated with the seed inoculant (Tables 5 and 6). The increased N fertilization effect in combination with the seed inoculant comparing to the control were significant, suggesting that increased N fertilizer in combination with *B. japonicum* inoculation facilitated greater nutrient content in the soybean fragments. Averaged across, the seed inoculant as a sub-plot significantly increased total N content in soybean root, shoot, leaf, grain and grain husk by 4.8%, 10.5%, 2.6%, 8.1% and 12.7%, respectively, as compared to the respective N fertilization treatment without the seed inoculation. Similar results were revealed on total P and N contents in the soybean vegetative and generative organs.

Table 4. N level in plant organs as affected by N fertilization and *B. japonicum* inoculation (%) (averaged across 2018 and 2019 growth seasons).

Treatments	N (%)				
	Root	Shoot	Leaf	Grain	Husk
Control	0.88d	0.51c	1.98d	3.55e	1.02e
Control+Br	0.92c	0.58bc	2.02c	3.66d	1.12e
$N_0P_{90}K_{60}$	0.97b	0.56bc	2.02c	3.77c	1.17d
$N_0P_{90}K_{60}$+Br	0.98b	0.61b	2.07b	3.88c	1.22d
$N_{30}P_{90}K_{60}$	1.12ab	0.64ab	2.08b	4.02bc	1.42b
$N_{30}P_{90}K_{60}$+Br	1.15a	0.68a	2.14a	4.56a	1.52a
$N_{60}P_{90}K_{60}$	1.02b	0.58bc	2.05b	3.77c	1.22d
$N_{60}P_{90}K_{60}$+Br	1.13ab	0.66a	2.11ab	4.22b	1.32 c

Means of three replications (n = 3) separated by lower case letters (a and e) in each column are significantly different at *P*≤5%.

Table 5. P level in plant vegetative organs as affected by *B. japonicum* inoculation and N fertilization (%) (averaged across 2018 and 2019 growth seasons).

Treatments	P (%)				
	Root	Shoot	Leaf	Grain	Husk
Control	1.66e	1.90d	1.32d	1.74e	1.18d
Control+ Br	1.74e	1.91d	1.39cd	1.76e	1.22c
$N_0P_{90}K_{60}$	1.82d	2.10c	1.41c	1.82d	1.24c
$N_0P_{90}K_{60}$+Br	2.00b	2.11c	1.43c	1.84d	1.24c
$N_{30}P_{90}K_{60}$	2.02b	2.86ab	1.56ab	2.04b	1.36b
$N_{30}P_{90}K_{60}$+Br	2.12a	2.91a	1.67a	2.14a	1.43a
$N_{60}P_{90}K_{60}$	1.91c	2.20b	1.51b	1.91c	1.30bc
$N_{60}P_{90}K_{60}$+Br	2.03b	2.89ab	1.62ab	2.08ab	1.36b

Means of three replications (n = 3) separated by lower case letters (a and e) in each column are significantly different at *P*≤5%.

While the addition of starter N fertilizer fulfils the immediate requirement of N of the legume at the germination, these practice combinations lead to higher availability of nutrients in the soil (Tarekegn and Kibret, 2017). An application of *B. japonicum* as a seed inoculant probably facilitated a proliferation of beneficial microbes, which might have created a favorable condition for plant growth. Notably, a significant N fertilizer x *B. japonicum* interaction increased soybean growth and development, positively affecting generative organs as well. That could also be interpreted based on positive roles of the bacterial strain on soil microbial activity, resulting in higher nutrient availability in the soil.

Table 6. K level in plant vegetative organs as affected by *B. japonicum* inoculation and N fertilization (%) (averaged across 2018 and 2019 growth seasons).

Treatments	K (%)				
	Root	Shoot	Leaf	Grain	Husk
Control	2.250d	2.325d	2.325d	2.700d	2.475e
Control+Br	2.325c	2.375c	2.375b	2.775c	2.550d
$N_0P_{90}K_{60}$	2.325c	2.400c	2.350c	2.775c	2.550d
$N_0P_{90}K_{60}$+Br	2.471bc	2.475b	2.400b	2.825b	2.625b
$N_{30}P_{90}K_{60}$	2.400c	2.550ab	2.450ab	2.925ab	2.625b
$N_{30}P_{90}K_{60}$+Br	2.750a	2.650a	2.500a	2.975a	2.700a
$N_{60}P_{90}K_{60}$	2.325c	2.500b	2.375b	2.850b	2.600c
$N_{60}P_{90}K_{60}$+Br	2.550b	2.525ab	2.475ab	2.950ab	2.650b

Means of three replications (n = 3) separated by lower case letters (a and e) in each column are significantly different at *P*≤5%.

A similar trend of the increase of nutrients in the soybean tissues depending on the combined N fertilizer x *B. japonicum* treatment was determined by analyses of chemical content (Tables 5, 6 and 7). It is well documented that plant uptake different macro and micronutrients in a balanced way. So, N applied in a higher dose increases demand for other nutrients, while accelerating plant growth and yield formation (Yucel et al., 2015). The soil with higher nutrient profiles is generally reflected in the plant vegetative parts' enhanced chemical content (N, P and K). Therefore, a higher amount of these nutrients were observed in the treated plants (Jahangir et al., 2021).

In this study, physiologic processes in soybean were suppressed when the plant had been treated with the higher rate of N fertilizer ($N_{60}P_{90}K_{60}$). This outcome shows that there is a need to reduce the detrimental effect of applied N in order to improve the N benefits to the legume crop and enhance N_2 fixation. In fact, soybean growth and nutrients uptake significantly increased with increasing fertilization rates, but the $N_{30}P_{90}K_{60}$ x *B. japonicum* treatment stabilized biomass ratio × plant yield interrelations. These results indicate that the combination of $N_{30}P_{90}K_{60}$ x *B. japonicum* can positively influence soil nutrient profiles and improve the uptake of nutrients by the plant as well as nutrient mobility within the plant. Despite the detrimental effect of N fertilization at the higher rate ($N_{60}P_{90}K_{60}$), the optimum level of N as starter fertilizer might increase the productivity of legumes with the seed inoculation.

Table 7: Soybean oil fatty acid compositions (g per kg) (averaged across 2018 and 2019 growth seasons).

Treatments	Palmitic acid (**$C_{16:0}$**)	Stearic acid (**$C_{18:0}$**)	Oleic acid (**$C_{18:1}$**)	Linoleic acid (**$C_{18:2}$**)	Linolenic acid (**$C_{18:3}$**)
Control	9.46c	1.71d	21.90c	52.73c	5.30d
Control+Br	9.88c	1.77d	21.81c	53.98b	7.72c
$N_0P_{90}K_{60}$	11.77b	1.95c	22.35c	54.12b	7.98c
$N_0P_{90}K_{60}$+Br	11.81b	1.92c	22.44	54.38b	8.34b
$N_{30}P_{90}K_{60}$	11.54b	2.53b	23.95b	55.76b	8.89a
$N_{30}P_{90}K_{60}$+Br	12.16a	3.62a	24.14a	56.01a	9.11a
$N_{60}P_{90}K_{60}$	12.06a	3.66a	24.34a	56.52a	9.05a
$N_{60}P_{90}K_{60}$+Br	12.18a	3.65a	24.43a	56.42a	8.62b

Means of three replications (n = 3) separated by lower case letters (a and c) in each column are significantly different at $P \leq 5\%$.

Nodulation, seed yield and seed quality

The nodulation in soybean roots increased with increasing N fertilization rate (Fig. 2). Also, the nodulation parallelly progressed with the growth stages of soybean. However, the nodulation rates were higher by 74.5%, 111.3%, 102.7% and 110.9% in the control treatment with the seed inoculation, $N_0P_{90}K_{60}$× *B. japonicum*, $N_{30}P_{90}K_{60}$×*B. japonicum*, $N_{60}P_{90}K_{60}$×*B. japonicum*, respectively as compared to the similar fertilization treatments without the inoculation. In overall, the nodule number was two-fold higher in soybean plants inoculated with the bacterial strain combined with the appropriate fertilization ($N_{30}P_{90}K_{60}$) rate (Fig. 2).

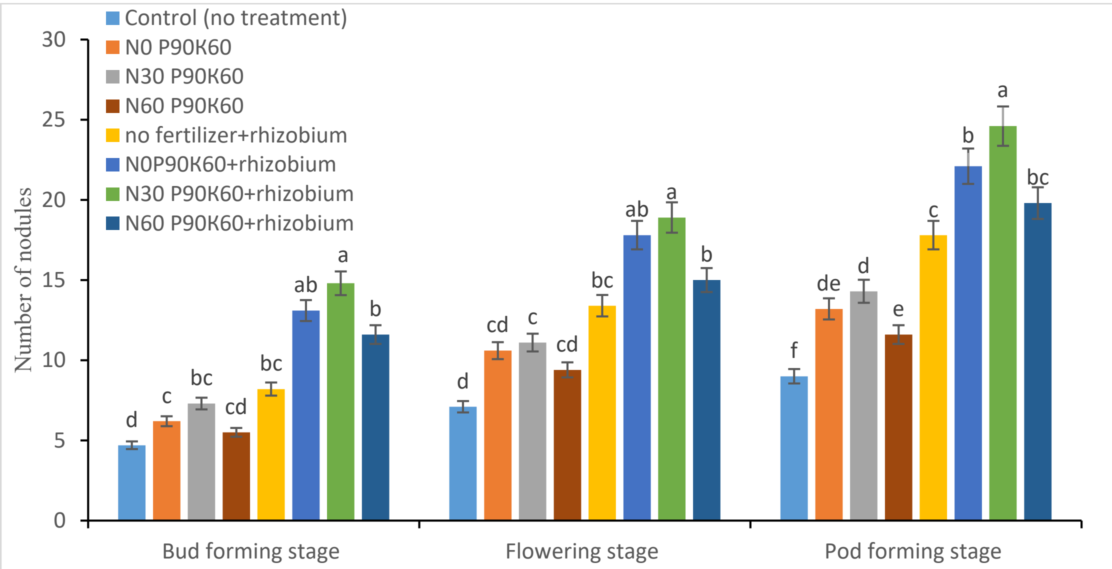

Figure 2. Quantity of nodules in the soybean root as affected by *B. japonicum* inoculation and N fertilization (averaged across 2018 and 2019 growth seasons). Bars show means of three independent replications (n = 3) separated by lower case letters (a and e) in each column are significantly different at $P \leq 5\%$.

A significant nonlinear response was detected when the soybean seed yield was trialed against N levels with and without the seed inoculant (Fig. 3). The soybean seed yield increased as the tested fertilization norms progressed in the seed inoculation treatments. However, an excessive N supply at a rate of $N_{60}P_{90}K_{60}$ with rhizobium inoculation had a negative effect on soybean yield. Results also showed that *Rhizobium* inoculation with $N_{30}P_{90}K_{60}$ fertilization treatment became the most effective, exhibiting the highest soybean seed yield (2.99 t per ha). In fact, the soybean seed yield was more pronounced with the seed inoculation. The soybean seed yield was higher by 20.4%, 19.0%, 34.1% and 6.1% in treatments of control (no fertilization) ×*B. japonicum*, $N_0P_{90}K_{60}$×*B. japonicum*, $N_{30}P_{90}K_{60}$×*B. japonicum*, $N_{60}P_{90}K_{60}$×*B. japonicum*, respectively as compared to the similar fertilization treatments without *Rhizobium* inoculation. In general, *B. japonicum* inoculation caused significant positive effects on soybean yield, whereas the excessive N supply had a negative effect on soybean yield as well (Fig. 3).

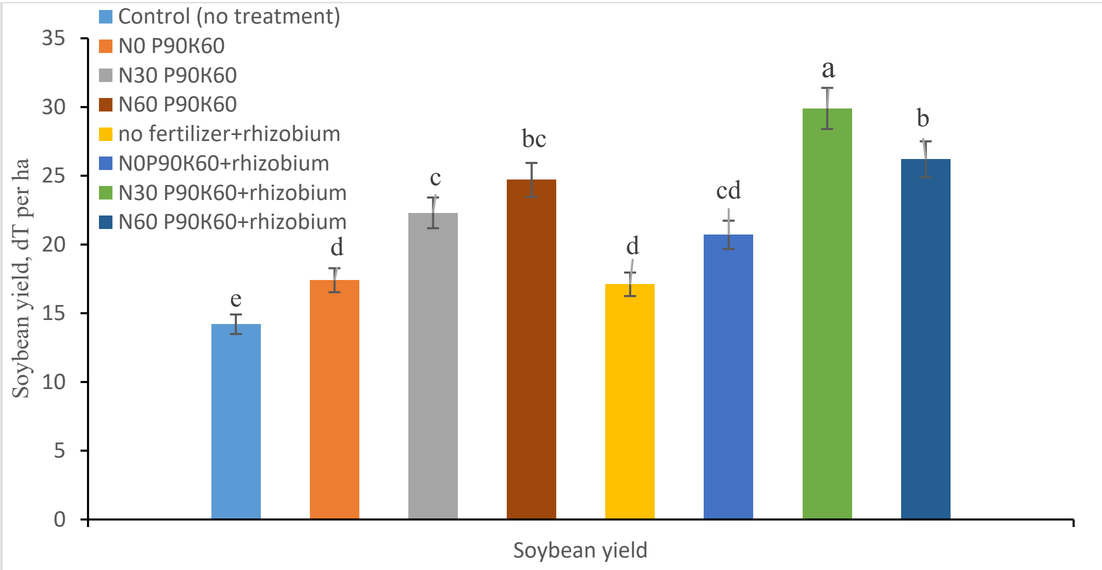

Figure 3. Soybean yield (dT per ha) as affected by N fertilization and *B. japonicum* inoculation (averaged across 2018 and 2019 growth seasons). Bars show means of three independent replications (n = 3) separated by lower case letters (a and e) in each column are significantly different at *P*≤5%.

Similarly, the seed oil and protein contents showed an increasing tendency with an increase in N fertilization level, which suggests that higher content of N is an essential factor for the soybean seed quality indicators (Figs. 4 and 5). In particular, the seed oil content was the highest when soybean treated with $N_{30}P_{90}K_{60}$ fertilization in combination with *B. japonicum* inoculation. This treatment ($N_{30}P_{90}K_{60}$×*B. japonicum*) increased the seed oil content by 26.2% compared to the control treatment. Likewise, the highest protein content was observed at the $N_{30}P_{90}K_{60}$×*B. japonicum* treatment (Fig. 4).

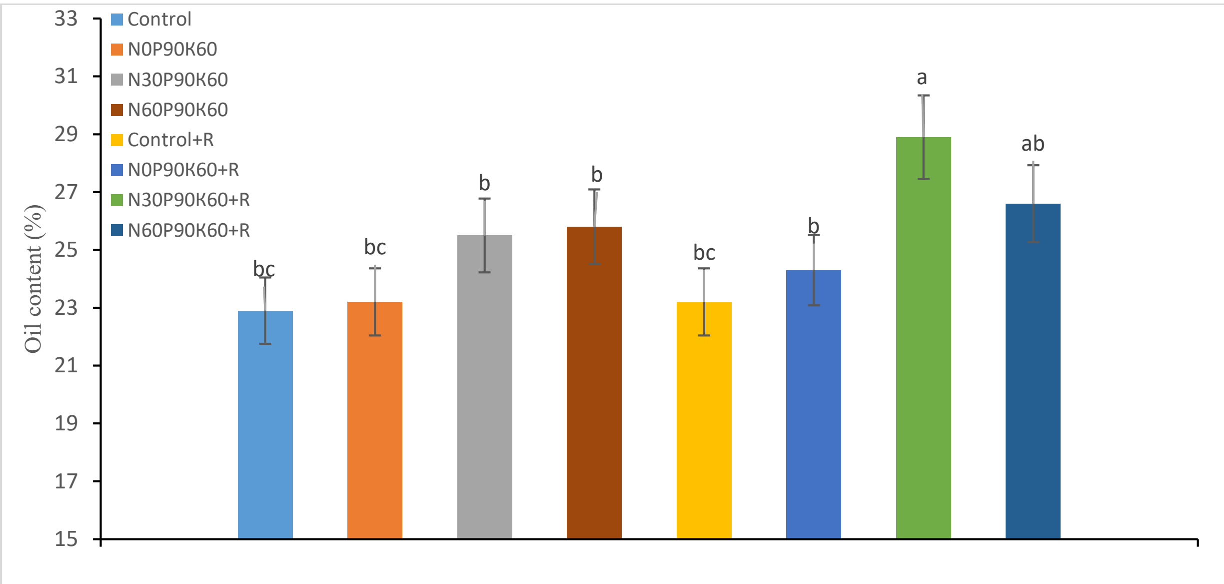

Figure 4. Soybean seed oil content (%) as affected by *B. japonicum* inoculation and N fertilization (averaged across 2018 and 2019 growth seasons). Bars show means of three independent replications (n = 3) separated by lower case letters (a and e) in each column are significantly different at *P*≤5%.

The most studied subject in soybean after being inoculated with *B. japonicum* dedicated to an increase in protein content. While recently some researchers reported about induced metabolic changes in soybean following inoculation with *B. japonicum* (Torres et al., 2018; Hungria et al., 2020; Abdel Latef et al., 2021; Soba et al., 2021). The results indicated that the combined use of *B. japonicum* inoculation with $N_{30}P_{90}K_{60}$ fertilization treatment increased nodule number per plant significantly as compared to the other treatments. But the higher rate of N fertilization ($N_{60}P_{90}K_{60}$) suppressed the nodulation process in soybean. Similarly, maximum values of soybean seed yield (2.990 t per ha) were observed by the combined application of $N_{30}P_{90}K_{60}$ fertilization with *B. japonicum* inoculation, indicating a synergistic effect of these chemical and biological factors.

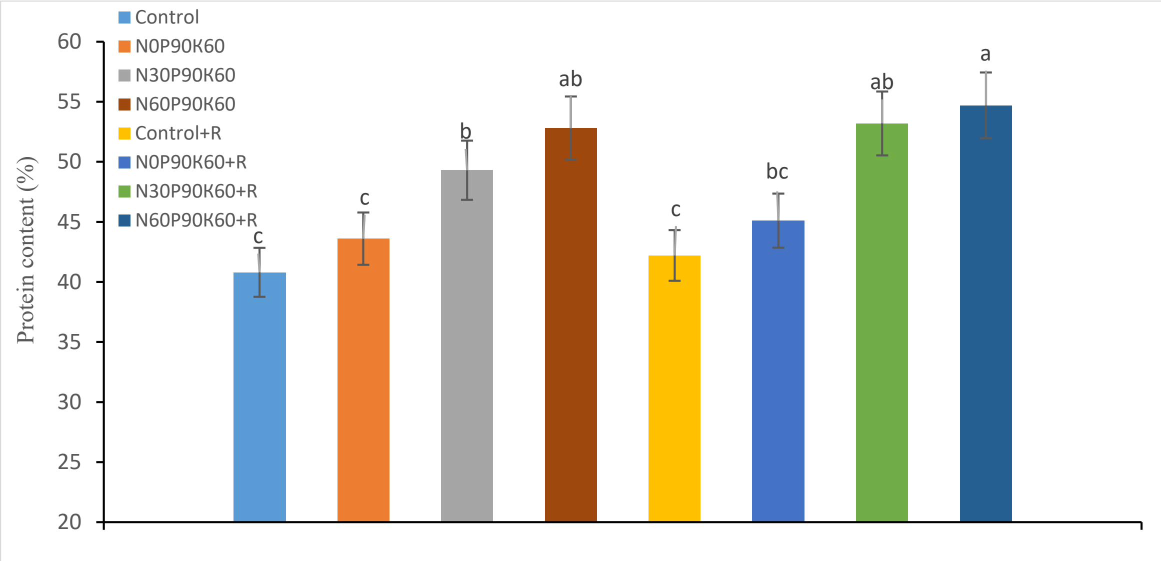

Figure 5. Soybean protein content (%) as affected by *B. japonicum* inoculation and N fertilization (averaged across 2018 and 2019 growth seasons). Bars show means of three independent replications (n = 3) separated by lower case letters (a and e) in each column are significantly different at $P \leq 5\%$.

The five essential fatty acids (palmitic, stearic, oleic, linoleic, and linolenic) were studied in the soybean seed oil under the eight fertilization treatments during the two growing seasons (Table 7). The predominant average content (56.52%) of linoleic acid was observed among all the fatty acids, whereas stearic acid showed the lowest average content (1.71%). As expected, the studied fatty acid contents increased with increasing N fertilization rate, while the $N_{30}P_{90}K_{60}$×*B. japonicum* combination exhibited a synergistic effect. An application of nitrogen at a rate of 60 kg per ha with the seed inoculant increased palmitic acid level by 28.7%, stearic acid level by 113.4%, oleic acid level by 11.5%, linoleic acid level by 6.99% and linolenic acid level by 62.6% as compared to the control. In most cases, the highest level of the studied fatty acids was observed at the $N_{30}P_{90}K_{60}$ fertilization treatment in association with *B. japonicum* inoculation, whereas no significant differences were found between the highest N rates with and without *B. japonicum* inoculation.

When the seed inoculant was applied, the higher rates of N (60 kg N per ha) fertilization significantly decreased the total seed yield of soybean. Previous reports have well discussed that high levels of inorganic N, especially nitrate N, inhibit nodule formation in legumes, plant growth, and economic soybean seed yield with a good quality product. Results of this study also showed that all fatty acids contents varied with highly significant differences between the highest and the lowest N rates, indicating N fertilization plays a key role in generating fatty acid composition. This variability in the seed quality is linked with the supply of nutrients due to the desirable N fertilization in conjunction with the beneficial bacteria strain as a seed inoculant (Zhang et al., 2014; Mourtzinis et al., 2017). Basically, one of the aims of improving legume seed quality is to improve growth condition through enhancing soil nutrients availability depending on their BFN ability in symbiosis with *Bradyrhizobium* (Gebauer et al., 2006; Khaitov et al., 2020). The enhanced seed oil, protein and fatty acid contents due to the specific agricultural practice are absolutely valuable indicators which are necessarily needed for healthier human nutrition (Chalon, 2006; Israilov et al., 2012). Despite the solid knowledge that N fertilization plays a major role in improving soybean seed composition, the current study points out the great contribution of the specific crop nutritional technology on improving seed quality parameters under saline aquifer.

CONCLUSIONS

This study showed the significance of using inorganic and organic amendments as an environmentally friendly approach for soybean production in saline soils. Despite soybean growth and nutrient content parameters of increased with increasing N fertilization rate, a synergistic effect of *B. japonicum* inoculation was substantial. In fact, a significant increase was observed in the seed oil, fatty acid and protein contents at the highest N fertilization treatment ($N_{60}P_{90}K_{60}$) with the seed inoculation. However, N fertilization at a rate of $N_{30}P_{90}K_{60}$ in conjunction with *B. japonicum* inoculation showed a balanced supply of nutrients for soybean growth and yield components. In addition, the proliferation of the bacterial strains in the soybean root was positively affected by *B. japonicum* combined with the adequate N supply, indicating the proper use of N might be helpful for bacterial existence. The highest yield and seed quality parameters were observed at the $N_{30}P_{90}K_{60}$×*B. japonicum* treatment which indicates this valuable agricultural practice might be used to the contribution of soybean production in saline areas.

ACKNOWLEDGEMENTS

This research was funded by the Internal projects of International Center for Biosaline Agriculture.

LITERATURE CITED

Abdel Latef, A.A.H., A.M. Omer, A.A. Badawy, M.S. Osman and M.M. Ragaey. 2021. Strategy of salt tolerance and interactive impact of Azotobacter chroococcum and/or Alcaligenes faecalis inoculation on canola (Brassica napus L.) plants grown in saline soil. Plants 10(1): 110.

Abdelghany, A.M., S. Zhang, M. Azam, A.S. Shaibu, Y. Feng, J. Qi, Y. Li, Y. Tian, H. Hong and B. Li. 2020. Natural variation in fatty acid composition of diverse world soybean germplasms grown in china. Agron.10: 24.

Abdiev, A., B. Khaitov, K. Toderich, K.W. Park. 2019. Growth, nutrient uptake and yield parameters of chickpea (*Cicer arietinum* L.) enhance by Rhizobium and Azotobacter inoculations in saline soil. J. Plant Nutr. 42(20): 2703-2714.

Allanov, K., K. Sheraliev, C. Ulugov, S. Ahmurzayev, O. Sottorov, B. Khaitov and K.W. Park. 2019. Integrated effects of mulching treatment and nitrogen fertilization on cotton performance under dryland agriculture. Commun. Soil Sci.Plant Anal. 50(15): 1907-1918.

Ashraf, M. and P.J.C..Harris 2013. Photosynthesis under stressful environments: An overview. Photosynth. 51: 163–190.

Asik, F.F. and H. Arioglu. 2020. The effect of rhizobium inoculation and nitrogen application on various agronomical and quality characteristics of peanut grown as a main crop. Turk J. Field Crops. 25(2): 100-106.

Azam, M., S. Zhang, J. Qi, A.M. Abdelghany, A.S. Shaibu, S. Ghosh, Y. Feng, Y. Huai and J. Gebregziabher. 2021. Profiling and associations of seed nutritional characteristics in Chinese and USA soybean cultivars. J. Food Compos. Anal. 98: 103803.

Aziz, I., T. Mahmood and K.R. Islam. 2013. Effect of long term no-till and conventional tillage practices on soil quality. Soil Tillage Res. 131: 28-35.

Cafaro La Menza, N., J.P. Monzon, J.E. Specht, J.L. Lindquist, T.J. Arkebauer, G. Graef and P. Grassini. 2019. Nitrogen limitation in high-yield soybean: Seed yield, N accumulation, and N-use efficiency. Field Crops Res. 237: 74–81.

Chalon, S. 2006. Omega-3 fatty acids and monoamine neurotransmission. Prostaglandins Leukot. Essent. Fat. Acids 75: 259–269.

da Mota R.P., R. De Camargo, E.M. Lemes, R.M.Q. Lana, R.F. de Almeida and E.R. de Moraes. 2019. Biosolid and sugarcane filter cake in the composition of organomineral fertilizer on soybean responses. Int. J. Recycl. Org. Waste Agric. 8(2): 131-137.

FAOSTAT. 2020. FAOSTAT database. Food Agric. Organ. UN. (accessed on 26 October 2022).

Gebauer, S.K., T.L. Psota, W.S. Harris and P.M. Kris-Etherton. 2006. n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am. J. Clin. Nutr. 83: 1526S–1535S.

Hammerschmiedt, T., J. Holatko, M. Sudoma, A. Kintl, J. Vopravil, P. Ryant and M. Brtnicky. 2021. Biochar and sulphur enriched digestate: utilization of agriculture associated waste products for improved soil carbon and nitrogen content, microbial activity, and plant growth. Agron. 11(10): 2041.

Horneck, D.A. and D. Hanson. 1998. Determination of potassium and sodium by flame emission spectrophotometry. Handbook of reference methods for plant analysis. 19: 153-155.

Htwe, A.Z., S.M. Moh, K. Moe and T.Yamakawa. 2019. Biofertilizer production for agronomic application and evaluation of its symbiotic effectiveness in soybeans. Agron. 9(4): 162.

Hungria, M., M.A. Nogueira, L.J.M. Campos, P. Menna, F. Brandi and Y.G. Ramos. 2020.Seed pre-inoculation with *Bradyrhizobium* as time-optimizing option for large-scale soybean cropping systems. Embrapa Soja-Artigo em periódico indexado (ALICE).

Ilker, E., M. Kocaturk, A. Kadiroglu, A. Yildirim, G. Ozturk, H. Yildiz, and I. Koken, 2018a. Adaptation Abilities and Quality Parameters of Selected Soybean Lines under Double Cropping in the Mediterranean Region. Turkish Journal of Field Crops 23 (1): 49-55. DOI: 10.17557/tjfc.421584

Ilker, E., M. Kocaturk, A. Kadiroglu, M. Altinbas, A. Yildirim, G. Ozturk and H. Yildiz. 2018b. Stability Analyses for Double Cropping in Soybean [(Glycine max L.) Merrill]. Turkish Journal of Field Crops 23 (2): 80-84.

Israilov, I., H. Atabaeva and N. Umarova. 2012. Soybean. Tashkent, Uzbekistan; TDAU University Press.

Jaga, P.K. and S.A. Sharma. 2015. Effect of biofertilizer and fertilizers on productivity of soybean. Ann. Plant Soil Res. 17(2): 171-174.

Jahangir, M.M., M. Jahiruddin, H. Akter, R. Pervin and K.R. Islam. 2021. Cropping diversity with rice influences soil aggregate formation and nutrient storage under different tillage systems. J. Plant Nutr. Soil Sci. 184(1): 150-162.

Januszewska A, E.M. Siedlecka, P. Glamowski and I. Tomasiak. 1999. Determination of oil content in rapeseeds using two methods–soxhlet extraction and pulsed nuclear magnetic resonance spectrometry. PN-EN ISO 659:1999.

Khaitov, B., J. Vollmann, J.Y. Pyon, K.W. Park. 2020. Improvement of salt tolerance and growth in common bean (*Phaseolus vulgaris* L.) by co-inoculation with native rhizobial strains. J. Agric. Sci. Tech. 22(1): 209-220.

Khan, S., A.E.L. Hesham, M. Qiao, S. Rehman and J.Z. He. 2010. Effects of Cd and Pb on soil microbial community structure and activities. Environ. Sci. Pollut. Res. 17: 288–296.

Kocaturk, M., P. Cubukcu, A.T. Goksoy, M. Sincik, E. Ilker, A. Kadiroglu, A. Yildirim. 2019. GGE biplot analysis of genotype× environment interaction in soybean grown as a second crop. Turk. J. Field Crops. 24(2): 145-154

Mahanta, D., R.K. Rai, S.D. Mishra, A. Raja, T.J. Purakayastha and E. Varghese. 2014. Influence of phosphorus and biofertilizers on soybean and wheat root growth and properties. Field Crops Res. 166: 1-9.

Marro, N., N. Cofré, G. Grilli, C. Alvarez, D. Lucas, D. Maestri and C. Urcelay. 2020. Soybean yield, protein content and oil quality in response to interaction of arbuscular mycorrhizal fungi and native microbial populations from mono- and rotation-cropped soils. Appl. Soil Ecol.152: 103575.

Mourtzinis, S., A.P. Gaspar, S.L. Naeve and S.P. Conley. 2017. Planting date, maturity, and temperature effects on soybean seed yield and composition. J. Agron. 109: 2040–2049.

NIAST. Methods of analysis of soil and plant. 2000. NIAST (National Institute of Agricultural Science and Technology) Suwon, Korea.

Nosheen, S., I. Ajmal and Y. Song. 2021. Microbes as biofertilizers, a potential approach for sustainable crop production. Sustainability 13: 1868.

Ozturk, E. 2021. Critical dose of nitrogen and phosphorus for the enhanced growth, yield, and quality components in mustard (Sinapsis Arvensis L.) In a semi arid environment. Turk. J. Field Crops. 26(2): 244-252.

Silva, L.R., C. Bento, A. Carolina Goncalves, J. David Flores-Félix, M. Helena Ramírez-Bahena, A. Peix and E. Velázquez. 2017. Legume bioactive compounds: Influence of rhizobial inoculation. AIMS Microbiol. 3: 267–278.

Soba, D., I. Aranjuelo, B. Gakière, F. Gilard, U. Pérez-López, A. Mena-Petite and A. Sanz-Saez. 2021.Soybean inoculated with one bradyrhizobium strain isolated at elevated [co2] show an impaired c and n metabolism when grown at ambient [CO_2]. Front. Plant Sci.12.

Son, T.T.N., V.V. Thu, L.H. Man, H. Kobayashi and R. Yamada. 2004. Effect of long-term application of organic and biofertilizer on soil fertility under rice-soybean-rice cropping system. Omonrice 12: 45-51.

SOYSTATS. 2020. Available online: http://soystats.com/international-world-vegetable-oil-consumption/ (accessed on 26 October 2021).

Stojsin M.M., S. Petrovic, M. Dimitrijevic, J. Šućur Elez, Đ. Malenčić, V. Zecevic and D. Knezevic. 2022. Effect of salinity stress on antioxidant activity and grain yield of different wheat genotypes. Turk. J. Field Crops 27(1): 33-40.

Sulieman, S., C.V. Ha, M.N. Esfahani, Y. Watanabe, R. Nishiyama, C.T.B. Pham, D.V. Nguyen and L.S.P. Tran. 2015. A promising new genetic resource for improved drought tolerance in soybean when solely dependent on symbiotic N2 fixation. BioMed Res. Int. 687213.

Szpunar-Krok, E., A. Wondołowska-Grabowska, D. Bobrecka-Jamro, M. Jańczak-Pieniążek, A. Kotecki and M. Kozak. 2021, Effect of nitrogen fertilisation and inoculation with bradyrhizobium japonicum on the fatty acid profile of soybean (*Glycine max* (L.) Merrill) Seeds. Agron. 11(5): 941.

Tarekegn, M.A. and K. Kibret. 2017. Effects of rhizobium, nitrogen and phosphorus fertilizers on growth, nodulation, yield and yield attributes of soybean at Pawe Northwestern Ethiopia. World Sci. News 67(2): 201-218.

Toker, C., J. Gorham and M.I. Cagirgan. 2009. Certain ion accumulations in barley mutants exposed to drought and salinity. Turk. J. Field Crops. 14(2): 162-169.

Torres, D., I. Benavidez, F. Donadio, E. Mongiardini, S. Rosas, S. Spaepen and F. Cassán. 2018. New insights into auxin metabolism in *Bradyrhizobium japonicum*. Res. Microbiol. 169(6): 313-323.

Wang, X., B. Liu, G. Wu, Y. Sun, X. Guo, Z. Jin, W. Xu, Y. Zhao, F. Zhang, C. Zou and X. Chen. 2018. Environmental costs and mitigation potential in plastic-greenhouse pepper production system in China: A life cycle asessment. Agric. Syst. 167: 186-194.

Wirasnita, R., T. Hadibarata, Y.M. Novelina, A.R.M. Yusoff and Z. Yusop. 2013. A modified methylation method to determine fatty acid content by gas chromatography. Bull. Korean Chem. Soc. 34(11): 3239-3242.

Yucel, D., C. Yucel, E.L. Aksakal, K. Barik, M. Khosa, I. Aziz, K.R. Islam. 2015. Impacts of biosolids application on soil quality under alternate year no-till corn-soybean rotation. Water Air Soil Pollut. 226(6): 1-11.

Zhang, J., Y. Ge, F. Han, B. Li, S. Yan, J. Sun and L. Wang. 2014. Isoflavone content of soybean cultivars from maturity group 0 to VI grown in northern and southern China. J. Am. Oil Chem. Soc. 91: 1019–1028.

Zimmer, S., M. Messmer, T. Haase, H.P. Piepho, A. Mindermann, H. Schulz, A. Habekuß, F. Ordon, K.P. Wilbois and J. Heß. 2016. Effects of soybean variety and Bradyrhizobium strains on yield, protein content and biological nitrogen fixation under cool growing conditions in Germany. Eur. J. Agron. 72: 38–46.