



Article Agronomic Performance of Soybean with *Bradyrhizobium* Inoculation in Double-Cropped Farming

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Abstract: Land degradation is a serious problem in arid regions, including in Central Asian countries. Soybean symbiosis with rhizobia microbes has an essential role in improving crop productivity and sustaining soil fertility in an arid environment. An experiment was conducted in light straw-colored sierozem soils in the Syrdarya region of Uzbekistan (41.4° N, 64.6° E) under arid conditions over the 2016–2017 and 2017–2018 growing seasons. This study aimed to assess the beneficial N fixation (BNF) ability of soybean in association with the Bradyrhizobium R6 strain and the Bradyrhizobium japonicum USDA110 strains and their combined effect on soil fertility and crop yield. The residues of winter wheat and soybean improved soil structure, i.e., soil humus and N and P contents, significantly differing from those on the soybean followed by summer fallow treatment. Furthermore, soybean in association with dual inoculation had the highest N derived from the atmosphere (Ndfa) $(62.9 \text{ kg N ha}^{-1})$, followed by individual soybean treatments with the R6 and USDA110 strains at 51.9 and 40.6 kg N ha⁻¹, respectively. Improved soil quality positively impacted crop output, increasing winter wheat and soybean yields by 36.5% and 34.6%, respectively. Likewise, the yield parameters, i.e., the number of pods, weight of grain per pods, and 1000 seeds were significantly higher in the inoculated treatment with the highest value observed in the dual-inoculated treatment. These results suggest the insertion of soybean with symbiotic bacteria into the cropping system has considerable potential to contribute to sustainable land management practices in arid zones.

Keywords: soybean; winter wheat; bradyrhizobium; N fixation; soil quality; salinity; yield

1. Introduction

Inappropriate crop rotation, excess use of agrochemicals, and soil mismanagement have led to the deterioration of land and water quality in Uzbekistan, dramatically deteriorating soil organic structure [1]. The implemented cotton–wheat rotation system further exacerbated the long-term problem, leading to a crop yield reduction of 20–30%. In addition to salinization, contamination by heavy metals and chemical compounds released by agriculture seriously diminished the soil's fertility, ecology, and physicochemical properties. As a result, the productivity of the agricultural sector has declined, significantly affecting the livelihoods of the population in this region, especially in rural areas.

Salinity and drought stresses are major challengers limiting crop production under the harsh environment of Uzbekistan. These two factors may cause great yield loss in grain legume productivity despite evolved tolerance mechanisms to abiotic and biotic stresses. Nevertheless, the symbiotic mutualism of legume and rhizobia could enhance resistance to abiotic and biotic stresses by promoting plant growth, nutrient uptake, and grain yield.



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Soybean (*Glycine max* L. (Merr)) is one of the recently introduced crops into the Uzbek agriculture and is driven by its abiotic and biotic stress tolerance, good adaptation, and high-quality grain characteristics. Apart from that, this crop in association with rhizobial strains facilitates the nitrogen fixation process, thereby improving soil structure and fertility even under extremely harsh environments. Microbial symbiosis is an important feature of legume crops; however, effective soybean production mostly depends on the appropriate use of inocula. This unique feature of soybean cultivation might facilitate and rejuvenate the low-input agricultural production of arid regions, while N fixation has great potential to maximize the profitability of the agrosystem.

The relationship between legumes and root symbiotic bacteria is vital to maintain a balance in terrestrial ecosystems, while biologically fixed N by this symbiotic relationship accounts for at least 70 million metric tons per year [2]. In addition to N fixation, some Rhizobium species promote plant growth with better nutrient uptake and disease suppression [3,4], functioning as the main component of sustainable agriculture. Recent studies also exhibited successful application of plant growth-promoting rhizobacteria (PGPR) for soybean growth, nutrients uptake, yield and seed quality as well as physicochemical properties of soil [5]. Meanwhile, an application of PGPR enhances plant productivity due to the production of phytohormones, enzymes, siderophores, exopolysaccharides, and other metabolites [6]. These beneficial functions of rhizobacteria are simultaneously reflected in reduced agrochemicals for crop production in the region [7].

Soybean cultivation as a second crop after winter wheat is not common in Uzbekistan, although it is an integral part of climate-smart and resource-efficient sustainable technologies. Water deficiency is considered as the main constraint in this region. Furthermore, insufficient knowledge and practices of farmers often cause land mismanagement, escalating food security problems and constraining biodiversity conservation in the region. However, the real challenges lie in the existing crop rotation and suitable crop diversity system in the region. This system requires agricultural interventions that enable the production of crops for food and feed through improving agronomic practices. These problems could be solved by introducing breakthrough innovations and implementing the most effective eco-friendly strategies such as better managing the relationship between legume and symbiotic bacteria and prudent usage of N fixation processes in crop production.

This study's hypothesis was the use of rhizobial inoculants for soybean cultivation grown as a second crop after winter wheat, thereby enhancing soil fertility and crop productivity under dryland agriculture of Uzbekistan. However, to date, this sort of research has been poorly conducted in this area, and there are many uncertainties. Therefore, this study was conducted with the objectives of focusing on soybean cultivation after winter wheat as an alternative source for improving soil fertility and crop production under Uzbekistan's arid conditions and identifying suitable bacterial inoculation for optimum grain yield and N_2 fixation.

2. Materials and Methods

2.1. The Study Area

The two-season (2016–2017 and 2017–2018 growing seasons) study was carried out in the light straw-colored sierozem soils in the Syrdarya region of Uzbekistan (41.4° N, 64.6° E) under arid conditions. The experiment site is situated 430 m above sea level with a predominantly harsh continental climate with 210–215 frost-free days.

Annual rainfall fluctuates at a range of 160–230 mm; the main part falls between late winter to early spring, without benefitting the annual crops (Table 1). An average daily temperature reaches 37 °C in July, and the lowest temperature is –2.3 °C observed in January. The rainfall amounts did not range substantially during the experimental years, except for the first year with an average 179.1 mm. The next two years, rainfall amounts were almost similar, averaging 194.1 mm—2017 and 192.4 mm—2018. According to the precipitation rates, 2016 was drier and warmer compared to the other experimental seasons. The annual evapotranspiration level usually reaches 2000 mm, making irrigation a necessity

for crop production. However, these climate variations are common in this region, without substantial damage to agriculture.

Table 1. Weather data on air temperature, rainfall, and relative humidity of the study area, Sirdarya region (2016 to 2018 growing years and long-term data).

		Month of the Year										
Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
					Air te	emperatur	e (°C)					
2016	1.2	3.8	6.9	12.4	25.7	23.4	25.6	29.3	29.3	13.6	6.8	1.5
2017	-4.0	-1.2	3.5	10.3	19.8	22.3	30.4	27.8	29.7	16.7	3.7	-2.5
2018	-2.5	0.3	4.9	11.2	20.6	27.9	30.1	29.9	26.8	15.9	2.9	-1.5
Long-term average (last 50 years)												
	-2.3	0.2	4.5	13.9	21.3	26.4	28.0	26.0	19.4	10.7	4.0	-1.7
Rainfall (mm)												
2016	35.5	33.6	30.2	16.8	20.4	1.7	0	0	3.2	7.5	8.4	21.8
2017	39.8	19.6	28.9	31.4	11.5	0.6	0	1.1	1.4	6.5	22.1	31.2
2018	32.5	20.9	26.8	29.8	16.4	1.2	0	0	1.0	9.1	21.5	33.2
	Long-term average (last 50 years)											
	36.1	28.7	31.4	27.4	16.2	1.4	0.0	1.1	3.0	8.7	19.4	29.6
					Relati	ve humidi	ty (%)					
2016	80	68	62	43	36	28	32	29	35	49	76	77
2017	79	73	65	36	43	33	30	27	39	47	69	82
2018	76	67	60	42	43	37	35	31	33	53	79	83
				L	ong-term a	average (la	ast 50 year	s)				
	81	74	65	45	44	36	33	28	36	51	75	80

Source: Meteorological Station of Sirdarya region.

The soil in this area is characterized by a poor structure and low organic matter, exhibiting 1.3–1.4 g/cm³ bulk density and 0.7–0.8% humus content (Table 2). The soil salinity was low (EC 2.5 dS m⁻¹), while pH showed neutral readings (6.8–6.9). The soil horizon at 0–30 and 30–50 cm depths contained total N 0.06–0.09 and 0.05–0.07%, total P 0.09–0.12 and 0.07–0.09 mg kg⁻¹, and exchangeable potassium 180–200 and 160–175 mg kg⁻¹, respectively. The ground water level is around 2.5–3 m depth, but it rises up to 1 m depth during the growing season, causing a secondary salination effect.

Table 2. Impact of soybean with rhizobium inoculation on soil physical and chemical structure.

	Soil Layer	Soil Bulk Density g/cm ³	Total g/kg			Mobile Forms mg/kg			
Ireatments	cm		Humus	Ν	Р	N-NO ₃	P_2O_5	K ₂ O	
	Beginning of the experiment (2016 spring)								
Before the experiment	0–30 30–50	$\begin{array}{c} 1.364 \pm 0.4 \text{ c} \\ 1.395 \pm 0.2 \text{ b} \end{array}$	7.50 e 7.31 f	0.61 d 0.47 e	0.88 c 0.71 e	6.06 b 4.32 c	30.0 с 28.8 с	180 a 160 c	
End of the experiment (2018 autumn)									
Winter wheat-Summer fallow	0–30 30–50	$\begin{array}{c} 1.385 \pm 0.7 \text{ b} \\ 1.449 \pm 0.4 \text{ a} \end{array}$	7.41 e 7.28 e	0.57 d 0.40 e	0.80 d 0.65 e	4.55 c 3.02 e	28.2 c 25.4 d	160 c 120 f	
Winter wheat-Soybean	0–30 30–50	$\begin{array}{c} 1.266 \pm 0.3 \text{ e} \\ 1.318 \pm 0.5 \text{ d} \end{array}$	8.25 b 7.70 d	0.84 b 0.70 c	0.95 b 0.80 d	5.25 b 6.12 b	31.4 c 29.0 c	160 c 150 d	
Winter wheat-Soybean + R6	0–30 30–50	$\begin{array}{c} 1.267 \pm 0.4 \text{ e} \\ 1.316 \pm 0.4 \text{ d} \end{array}$	8.27 b 7.70 d	0.81 b 0.57 d	0.90 c 0.85 c	6.55 a 4.02 d	27.0 c 21.0 d	160 c 140 e	
Winter wheat-Soybean + USDA110	0–30 30–50	$\begin{array}{c} 1.243 \pm 0.4 \text{ f} \\ 1.308 \pm 0.6 \text{ d} \end{array}$	8.31 b 7.80 d	0.84 b 0.60 d	1.00 b 0.90 c	6.62 a 3.07 e	37.8 b 28.2 с	160 c 150 d	
Winter wheat-Soybean R6 + USDA110	0–30 30–50	1.226 ±0.4 h 1.367 ±0.3 c	8.50 a 8.10 c	1.05 a 0.90 b	1.15 a 1.00 b	6.72 a 4.05 c	46.0 a 27.6 c	180 a 170 b	

Means marked with different letters represent significant differences (p < 0.05).

2.2. Experiment Design

The experiment was set in Fall 2016 and arranged as a split-plot design with three replicated blocks. Some 1 m-wide buffers were maintained between blocks and plots to avoid any chance of contamination. The main factor was winter wheat and soybean rotated cultivation system. Subplots included three rhizobial treatments plus control. The main plot size was 24 m (width) by 36 m (length), and the subplot size was 4 m (width) by 12 m (length). A row space was 0.5 m, consisting of eight rows per plot. Data collection was not conducted in the two outer rows to exclude external effects, and the six internal rows were subjected to plant and soil sampling.

The soybean cultivar 'Orzu' was planted at a seeding rate of 60 kg ha⁻¹, and the winter wheat cultivar 'Turon' was seeded at rate of 200 kg ha⁻¹. After planting winter wheat in October, the experiment area was irrigated with a norm of 1100–1200 m³ hectare⁻¹ by surface furrow irrigation. Irrigation was conducted two to three more times depending on the climate in the next year's spring with the same norm. Winter wheat was harvested in the middle of June in both years. Soybean was planted in the second part of June. Soybean was irrigated three times with approximately 950–1050 m³ ha⁻¹ at each irrigation and equally for all experiment plots during the vegetation season. This early-ripening soybean variety was harvested in the middle of October each year.

Inorganic sources of N, P, and K in the forms of ammonium nitrate (34%), superphosphate (17%), and muriate of potash (52%) were applied in the experimental plots. Recommended doses of N, P (P₂O₅), and K (K₂O) were 200:140:140 kg ha⁻¹ for winter wheat and 100:60:40 kg ha⁻¹ for soybean. These fertilization applications were split into three portions, allowing better nutrient uptake of each crop.

Agronomic measures developed during many years for this region such as weeding, plant protection, and irrigation were delivered equally to all plots.

2.3. Chemical Analysis of Soil and Plant Samples

Soil samples collected at 0–30 and 30–50 cm soil layers in sealable plastic bags at the beginning and end of the experiment were subjected to chemical analysis. The soils were air-dried for two weeks at room temperature, ground, and sieved through a 2 mm mesh.

Soil salinity and alkalinity were determined with pH and EC meters at a 1:5 ratio of soil and distilled water as described in the USDA method [8]. The mixture of 10 g of soil and 50 mL of distilled water in a 100 mL beaker was shaken by hand every half hour for 30 s. After, the extract was obtained, and the EC was measured by a conductivity meter (WTW, Cond 315i), and the pH readings were taken by a pH meter (Oakton pH 5).

Soil physical characteristics were analyzed with standard methods developed by Ryan, Estefan, and Rashid [9]. In addition, the distillation method proposed by Tyurin and Lancaster [10] was used to detect soil organic matter [11].

The total nitrogen difference (TND) method was used in calculating N fixed by soybean from the atmosphere (Ndfa). In this method, Ndfa was determined by deducting the total N of the nonfixing (noninoculated) soybean from the total N accumulated by the nitrogen-fixing (inoculated) soybean [12].

Ndfa TND (kg ha⁻¹) = N acc Fixing Soybean (kg ha⁻¹) – N acc Nonfixing Soybean (kg ha⁻¹). Using the TND method, the N balance in Nfix of the whole plant, i.e., shoots and sampled roots, was calculated.

2.4. Rhizobial Strains

Bradyrhizobium R6 and *Bradyrhizobium japonicum* USDA110 strains were maintained on yeast mannitol agar (YMA) slants inside a freezer at a temperature of -80 °C as proposed by Somasegaran and Hoben [13] at the Biotechnology Department of Tashkent State Agrarian University, Tashkent, Uzbekistan.

The bacterial strains were cultured in a Yeast Extract Mannitol broth (YEM) medium inside a shaker at 28 °C. A spectrophotometer was used to detect the bacterial strains'

multiplication process every after 24, 48, and 72 h. The bacterial strains were grown until an approximate 10⁹ CFU mL⁻¹ concentration.

Before inoculation of soybean seeds, sterilized farmyard manure as a carrier material was separately mixed with the selected strains at a 1:1 proportion (100 g carrier material and 100 mL suspension). After sterilization of soybean seeds with 10% sodium hypochlorite solution for 5 min, these seeds were thoroughly washed with sterile, distilled water. Then, the soybean seeds were mixed with the bacteria carrier composition under shade directly before sowing. In the case of coinoculation, the test strains were mixed in equal proportion and further mixed with the carrier material.

2.5. Plant Growth and Yield Components

Three plants from each plot were randomly selected and sampled at the flowering stage. The fresh plant samples were oven-dried at 65 °C for 72 h until reaching a constant weight. Total nitrogen in plant samples was determined by the Kjeldahl method [14]. Phosphorous content was analyzed by the standard HClO₄ digestion method proposed by Bowman [15], whereas potassium was identified by a flame photometer (model Carl-Zeiss, Jena, Germany) as described by Horneck and Hanson [16] and calculated as percentages.

Wheat grain yield was measured at 1 m^2 in each plot after harvesting by hand when the standard moisture content was 14% of fresh weight. Before harvesting, spike length and weight were detected from three points in each plot. Accordingly, the average number of spikes per m², grains per spike, and weight of 1000 seeds were determined by counting. Root residues amounts were determined on 1 square meter after digging out available roots from three points in each plot, and then the average ton per hectare was calculated. Accordingly, the weight of clean, dry residues was expressed on a per hectare basis.

2.6. Statistical Analysis

Data obtained during the two-season experiment were subjected to analysis of variance with Anova (CropStat) program (International Rice Research Institute, Los Baños, Philippines). Winter wheat and soybean cultivars and rhizobia strains were considered as fixed effects, while random effects were replication (block), year, and interactions with year. The year effect was not significant, which allowed for pooling the values before the statistical analysis.

3. Results

3.1. Impact of Soybean Cultivation with Rhizobium Inoculation on Soil Structure

Soil physical and chemical structures were significantly higher when soybean was cultivated with dual inoculation after winter wheat compared to other treatment combinations (Table 2). Soil 0–30 and 30–50 cm layers exhibited 1.364 and 1.395 g cm⁻³ soil bulk density, respectively, at the beginning of the experiment. Slight soil compaction with values of 1.385 and 1.449 g cm⁻³ was detected at 0–30 and 30–50 cm soil layers, respectively, under the trial of winter wheat with a subsequent summer fallow. Soybean cultivation after winter wheat decreased soil bulk density considerably in all treatments, regardless of rhizobium inoculation. However, a decrease in soil bulk density was more pronounced in the bacterial inoculated treatments, especially under the coinoculated treatments ranging between 1.226 and 1.367 g cm⁻³.

As expected, soybean grown after winter wheat contributed to enhancing residue input in the soil, although the highest indicators were received after the coinoculation measures (Table 3).

	W	inter Wheat			Total Straw		
Treatments	Straw Residue ton/ha	Root Residue ton/ha	Total ton/ha	Straw Residue ton/ha	Root Residue ton/ha	Total ton/ha	Residues ton/ha
Winter wheat-Summer fallow	0.78 d	2.15 d	2.93 d	-	-	-	2.93 d
Winter wheat-Soybean	0.83 c	2.67 c	3.50 c	0.22 b	1.04 c	1.26 c	4.76 c
Winter wheat-Soybean + R6	0.96 b	2.65 c	3.61 b	0.25 b	1.20 b	1.45 b	5.06 b
Winter wheat–Soybean + USDA110	0.98 b	2.73 b	3.71 b	0.25 b	1.23 b	1.48 b	5.19 b
Winter wheat–Soybean R6 + USDA110	1.16 a	3.25 a	4.41 a	0.45 a	1.43 a	1.88 a	6.29 a

Table 3. Straw and root residues (averaged across 2016–2017 and 2017–2018 growing seasons).

Means marked with different letters represent significant differences (p < 0.05).

Rhizobium inoculations significantly enhanced soybean residue weights as well as total residues. Soybean cultivation after winter wheat yielded 4.76 t ha⁻¹ total residues, exhibiting a 62.5% increase over the winter wheat–summer fallow treatment. While soybean inoculation with R6 and USDA110 strains showed further increases of 6.3 and 9.03%, respectively, as compared to the winter wheat–soybean without inoculation treatment, the difference was not significantly different among the rhizobium-inoculated treatments. The greatest value of total residue mass was observed when soybean was coinoculated with the tested strains, showing a 114.7% increase (6.29 t ha⁻¹) as compared to the winter wheat–summer fallow treatment.

Taken together, the tested crops' residues impacted positively on the soil's physical and chemical parameters, although the highest values were observed in the treatment of soybean cultivation with rhizobium coinoculation.

3.2. Nutrients Returned to the Soil with the Residues of Wheat and Soybean

Soybean cultivation with the inoculated bacterial strains resulted in higher N, P, and K amounts returned to the soil by the crop residues than those in the control treatment (Table 4). According to the results, soybean cultivation after winter wheat substantially enhanced the amount of nutrients returned to the soil by 128.4%; 134.1%, and 228.2% for N, P, and K, respectively.

Table 4. Nutrients returned to the soil by crop residues and Ndfa (averaged across 2016–2017 and 2017–2018 growing seasons).

Treatments	Nutrients Returned with Winter Wheat Residues kg/ha			Nutrients Returned with Soybean Residues kg/ha			Ndfa	Total Nutrient Amount kg/ha		
	Ν	Р	К	Ν	Р	К	ку/па	Ν	Р	К
Winter wheat–Summer fallow Winter wheat–Soybean Winter wheat–Soybean + R6	18.3 d 21.7 c 22.4 b	8.2 d 9.4 c 10.2 b	10.3 d 12.9 c 13.4 b	20.1 c 23.9 b	9.8 b 9.7 b	20.9 b 20.7 b	- 40.6 c	18.3 e 41.8 d 86.9 c	8.2 d 19.2 c 19.9 c	10.3 c 33.8 b 34.1 b
Winter wheat-Soybean + USDA110 Winter wheat-Soybean R6+ USDA110	22.4 b 29.2 a	10.8 b 11.9 a	13.4 b 16.3 a	24.4 b 27.5 a	9.9 b 11.3 a	21.2 b 24.2 a	51.9 b 62.9 a	98.7 b 119.6 a	20.7 b 23.2 a	34.6 b 40.5 a

Means marked with different letters represent significant differences (p < 0.05).

N₂ fixation efficiency (Ndfa) was significantly greater in the soybean coinoculated treatment followed by individual treatments with the USDA110 and R6 strains. These values were eventually reflected in the nutrient amounts returned to the soil. The use of R6 exhibited 374.9% higher N, 142.7% higher P, and 231.1% higher K in the residues of the tested crops as compared to the control treatment. The application of USDA110 for soybean inoculation significantly enhanced the N, P, and K contents by 439.4%, 152.4%, and 235.9% as compared to the control treatment. Furthermore, the combined use of R6 and USDA110 further exerted the beneficial effect, resulting in 553.6% higher N, 182.9% higher P, and 293.2% higher K when compared with the control treatment. Particularly, the

treatment with the coinoculation of R6 and USDA110 was found to be the most effective for returning P, N, and K nutrient elements to the soil.

3.3. Impact of Soybean with Rhizobium Inoculation on Crops Yield

Both crops produced high yields due to soybean cultivation as a follow-up crop (Tables 5 and 6). Moreover, soybean inoculation with the tested *Rhizobium* strains further enhanced the beneficial effect. The results show that soybean cultivation significantly increased wheat growth and yield parameters, i.e., grain weight per spike by 18.8%, 1000 seed weight by 3.1%, straw biomass by 12.1%, and grain yield by 18.7% as compared to the control.

Table 5. Straw biomass and grain yield of winter wheat (averaged across 2016–2017 and 2017–2018 growing seasons).

Treatments	Straw Biomass dT/ha	Grain Weight per Spike g	Weight of 1000 Seeds g	Grain Yield dT/ha	
Winter wheat-Summer fallow	$75.4\pm0.6~\mathrm{d}$	1.6 c	42.5 c	$50.4\pm0.8~{ m d}$	
Winter wheat-Soybean	$84.5\pm1.1~{\rm c}$	1.9 b	43.8 b	$59.8\pm0.1~{ m c}$	
Winter wheat–Soybean + R6	87.6 ± 1.2 b	1.9 b	44.5 a	65.4 ± 0.4 b	
Winter wheat-Soybean + USDA110	$89.4\pm1.3~\mathrm{b}$	2.0 a	44.9 a	$66.2\pm\!0.5\mathrm{b}$	
Winter wheat–Soybean R6 + USDA110	$91.5\pm1.2~\mathrm{a}$	2.0 a	45.2 a	68.8 ±0.2 a	

Means marked with different letters represent significant differences (p < 0.05).

Table 6. Soybean yield (averaged across 2016–2017 and 2017–2018 growing seasons).

Treatments	Number of Pods per Plant Piece	Weight of Grain per Pod g	Weight of 1000 Seeds g	Soybean Yield dT/ha	
Winter wheat–Summer fallow	-	-	-	-	
Winter wheat-Soybean	$43.0\pm0.7~\mathrm{c}$	0.29 c	95 c	$21.7 \pm 0.2 \text{ d}$	
Winter wheat-Soybean + R6	46.6 ± 0.6 b	0.30 b	101 b	$24.9 \pm 0.6 \mathrm{~c}$	
Winter wheat–Soybean + USDA110	$47.0\pm0.7~\mathrm{b}$	0.31 b	102 b	$26.9\pm\!\!0.6\mathrm{b}$	
Winter wheat-Soybean R6 + USDA110	50.1 ± 0.6 a	0.35 a	105 a	29.2 ±0.4 a	

Means marked with different letters represent significant differences (p < 0.05).

Individual application of R6 and USDA110 further enhanced the yield attributes of wheat, indicating 18.8–25.0% higher grain weight per spike, 4.7–5.6% higher weight of 1000 seed, 16.2–18.6% higher straw biomass, and 29.8–31.3% higher grain yield, respectively than those in the control treatment.

Furthermore, the coinoculation of these bacterial strains surpassed the individual applications. The coaddition of the bacterial strains substantially increased grain weight per spike by 25%, weight of 1000 seed by 6.4%, straw biomass by 21.4%, and grain yield by 36.5% as compared to the control treatment values.

A similar increasing trend of yield attributes due to individual and especially combined inoculations was observed in soybean. The coinoculation of the tested strains was very effective for yield parameters, enhancing the number of pods by 16.5%, number of grains in each pod by 14.3%, pod weight by 20.7%, weight of 1000 seeds by 10.5%, and grain yield by 34.6% as compared to the noninoculation treatment.

Overall, the coinoculation of soybean with the bacterial strains proved very effective in improving yield attributes and crop biomass, which was reflected in the nutrients returning to the soil in the form of residues and could be the best choice for enhancing agricultural inputs.

4. Discussion

Having intercropped with grain legumes, a follow-up crop benefits from enhanced soil N and organic matter, which in turn may result in vigorous plant growth and high yield [17,18]. Apart from these advantages, this cropping system reduces the amount of chemical fertilizers, impacting positively on long-term agricultural sustainability [19]. In light of this background, the field experiments were maintained for the two seasons of double cropping with winter wheat followed by early-ripening soybean.

Returning root and shoot residues to the soil might be the main reason for the improvement of soil bulk density and simultaneously of soil physical and chemical structures. The softening of soil bulk density is directly associated with soil biological processes, i.e., aeration, mineralization, and bacterial and enzyme activities. On the other hand, soil physical structure significantly deteriorated under the winter wheat plus summer fallow treatment with enhancing soil bulk density. Moreover, soybean cultivation after winter wheat significantly improved soil humus and N and P contents. Especially, *Rhizobium* inoculations of soybean tended to increase considerably the above-mentioned soil quality parameters compared to the control treatment, but the highest values were observed under the coinoculated treatment. There were recent studies showing crop residue input at the upper depth of soil have many advantages, including slowing the nutrient mineralization process, controlling salt accumulation, and enhancing salt leaching [20,21]. The knowledge on nutrients returned to the soil as crop residues and improved soil physical-chemical properties is vital to maintain a balance within soil biological processes as well as to optimize crop yield [22].

There are many requirements for isolates of *Rhizobium* and *Bradyrhizobium* genera to be used for the inoculation of legumes such as well nodulation, N fixation, tolerance to abiotic and biotic stresses. These characteristics are a great asset for the use of rhizobium inoculation for soybean production under low-input production systems as the main source of N. The winter wheat followed by summer fallow treatment accumulated the lowest quantity of N (18.3 kg N ha⁻¹) due to the lowest root and shoot residues left in the soil. However, the N accumulation substantially enhanced up to 41.8 kg N ha⁻¹ when soybean cultivation was arranged after winter wheat. The bacterial strains' individual application promoted the N accumulation process, indicating 86.9 and 98.7 kg N ha⁻¹ for R6 and USDA110, respectively. The highest the N accumulation (119.6 kg N ha⁻¹) was obtained after coinoculation of the bacterial strains. This is explained by the fact that 47 to 53% of this nitrogen was mobilized from the atmosphere, while other parts came from shoot and root residues.

Soybean plants inoculated with the bacterial species had higher biomass production; under the coinoculated treatment the effect was especially obvious. Likewise, the soybean yield parameters, i.e., number of pods, weight of 1000 seeds, and grain yield were significantly higher in the inoculated treatments, with the highest value observed in the dual-inoculated treatment. Compared to the winter wheat–soybean treatment, the bacterial inoculations increased soybean yield by 14.6, 23.9, and 34.6% under R6, USDA110, and R6 + USDA110 strain usage, respectively These results suggest that using microbial inoculants as an eco-friendly approach can nourish soils by enhancing nutrient availability, thereby enhancing the efficiency within agriculture cropping systems. Considerable difference of plant performance is highly likely associated with the improvement of nutrition under the soybean inoculated treatments and the strong dependence of the plant species on nutrient availability [23]. This proactive approach may substitute chemical fertilizers usage with different combinations of microbial inoculations [24].

Inoculated soybean cultivation triggered biomass accumulation and yield attributes in wheat as well. For instance, the yield of succeeding winter wheat increased by 18.7%, 29.8%, 31.1%, and 36.5%, respectively, after noninoculated soybean, soybean + R6, soybean + USDA110, and coinoculated soybean cultivation. Similarly, in recent years, several researchers pointed out the positive impact of legumes on enhancing the productivity of subsequent crops by the increased availability of nutrients [25,26]. Our work supports the development of an integrated crop rotation system with microbial application that provides efficient nutrients and water management, enhances soil quality and crop yield, and maintains sustainable agricultural management. The improvement of the double cropping systems with the ambition to push forward the concept of biological approaches, such as the formulation and use of new efficient inoculants, can be very profitable and significantly increase crop productivity in arid regions [27,28]. The introduction and scaling up of climate-smart agrotechnologies require a huge effort especially under harsh environments, but these spent resources will pay off in the long term.

5. Conclusions

This study showed that soybean cultivation with rhizobium inoculation after winter wheat is an effective strategy that improves basic soil productivity and alleviates food security-related challenges. Under the coinoculated bacterial treatment of soybean, the agronomic performances of these two tested crops were higher than any of the tested treatments, exhibiting an apparent positive effect of this arrangement in arid conditions. Moreover, coinoculated soybean greatly enriched soil nutrient (NPK) content, while gradually increasing the fertility of soil prone to salt stress.

The potential of rhizobium coinoculation in soybean production is a vital indicator for the recommendation and adoption in degraded soils of Uzbekistan that might be inserted into the double-cropped farming system to optimize agricultural production in arid regions.

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