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Synergistic Effect of PGPR and Nutrient Complex on Soybean Seed Germination and Initial Seedling Growth

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Abstract

Biostimulants based on *Bradyrhizobium japonicum* are commonly used in soybean production. However, the effect of nitrogen-fixing bacteria in consortia with other plant growth-promoting rhizobacteria (PGPR) and their integration with mineral nutrients on soybean seed quality has not been explored. The study aimed to examine the effects of five treatments on seed germination and initial seedling growth of two soybean cultivars ('NS Apolo', 'NS Rubin'): control (untreated seeds); *Br. japonicum* (BJ), BJ and nutrient complex (NC), BJ, *Azotobacter chroococcum* (AC), *Bacillus subtilis* (BS), and NC; BJ, AC, *Bacillus megaterium* (BM), and NC. Seed treatments significantly enhanced germination energy, seedling vigor index, root length, fresh shoot weight, fresh root weight, dry shoot weight, and dry root weight of both cultivars, as well as final germination, shoot length, and shoot elongation rate of 'NS Rubin', as compared to the control. The highest effect on the investigated parameters was achieved by integrated use of PGPR and nutrients (BJ + BM + AC + NC), indicating that integration of PGPR with a targeted NC represents an innovative approach with practical implications for improving early soybean establishment and field performance.

Keywords: *Azotobacter*; *Bacillus*; *Bradyrhizobium*; *Glycine max*; micronutrients; seed treatment; seed quality and performance; PGPR

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1. Introduction

Soybean (*Glycine max* (L.) Merrill) is a major leguminous crop cultivated worldwide, valued for its high-quality plant-based protein and oil content, as well as other valuable nutrient compounds such as amino acids, dietary fiber, various vitamins, minerals, and antioxidants [1]. Due to its ability to establish symbiotic relationships with nitrogen-fixing bacteria, soybean cultivation provides multiple environmental and economic benefits in sustainable agriculture, including reducing reliance on synthetic fertilizers and greenhouse gas emissions, improving overall soil health and fertility, and increasing biodiversity [2].

Uniform seed germination and vigorous initial seedling growth are vital for optimal soybean establishment, productivity, and resilience to abiotic and biotic stresses. Main problems in soybean seed production include environmental stressors, such as extreme drought, high temperature and high moisture [3]. The soybean plants are vulnerable to abiotic stresses in reproductive stages critical for seed development (R1–R6), particularly during

the flowering and pod development, when stress can cause a decrease in seed number, size and weight, also affecting seed quality and yield potential [4]. Furthermore, diseases caused by seed-borne fungi (e.g., seed rot and seedling blight, *Fusarium* spp.; phomopsis seed decay, *Diaporthe*/*Phomopsis* complex) can significantly reduce seed viability [5,6]. In addition, inadequate conditions during storage can contribute to seed deterioration and vigor loss or evoke pathogen growth [7]. The challenges posed by global climate change further exacerbate these obstacles to soybean production, potentially leading to serious crop failures and significant yield reductions [8].

Plant growth-promoting rhizobacteria (PGPR) are a group of beneficial bacteria widely known for their ability to enhance plant growth and health. The mechanisms behind plant growth-promoting (PGP) actions involve biological nitrogen fixation (BNF), solubilization of soil nutrients (e.g., P, K, Zn), production of plant hormones (auxins, gibberellins, cytokinins), and biocontrol of plant pathogens [9]. The key PGPR genera, namely *Bradyrhizobium*, *Bacillus*, and *Azotobacter*, comprise the most commercially utilized inoculants in agricultural production. Symbiotic and PGP performance of PGPR-based inoculants depends on their survival, colonization, and interaction with the plants. Due to their variable efficacy in the field, it is necessary to develop novel formulations of PGPR inoculants which will comprehend all mechanisms and interactions of bacteria and plants, as well as adverse environmental factors that restrict their wider application [10]. One of the most promising approaches is co-inoculation of PGPR on legumes, as well as transferring PGPR formulations from a single bacterial strain or species to consortia of more strains that belong to different species [11].

In order to achieve optimal growth and development leading to high and stable yields, soybean demands a sufficient supply of nutrients. At the stage of seed germination and initial growth of soybean seedlings, the most important nutrients are phosphorus (P), nitrogen (N), potassium (K), calcium (Ca), sulfur (S), and magnesium (Mg). Additionally, microelements that support soybean germination are zinc (Zn), manganese (Mn), copper (Cu), molybdenum (Mo), and iron (Fe) due to their role in enzymatic reactions, metabolism and development of roots and shoots [12,13]. Nutrient complexes contain essential macro- and micronutrients in bioavailable forms. These nutrients support key functions such as enzymatic activation, energy metabolism, and the synthesis of nucleic acids and proteins [14,15]. Thus, nutrient complexes may have an important role in modulating physiological and biochemical processes during germination and the initial stages of plant development [14,15]. The presence of nutrients mentioned above is crucial for triggering metabolic processes that promote cell division, elongation, and differentiation of roots and shoots [14,15]. When used in combination with PGPR, which can promote nutrient solubilization, phytohormone production, and stress resilience, a synergistic effect may emerge. This synergistic interaction is particularly valuable for legumes such as soybean, which depend on both external nutrient sources and microbial partnerships for optimal early growth and development. Mineral nutrients are also important determinants of effective symbiosis between legume host and rhizobia, considering that symbiotic bacteria require different macro- and microelements for invading and colonizing the root systems [16]. For example, an optimal amount of essential nutrients (N, P, and Mg) stimulates soybean growth, root architecture, root colonization by *Br. japonicum*, nodule formation, and nitrogen accumulation in plant tissues [17]. The colonization and functional traits of *Bacillus* and *Azotobacter* strains can also be promoted by nutrient supplements, especially considering the intense competition for niches and nutrients that occurs among introduced and indigenous rhizosphere microflora.

Several previous studies have reported strong improvements in seed germination, seedling vigor, and early growth of soybean when applying PGPR alone or in co inoculations [18,19]. Although benefits of PGPR are well-known, only a few studies have

addressed their combined effects with nutrient amendments, particularly during the early stages of plant growth and development [20]. In particular, studies on how PGPR consortia respond to integration with nutrient complex and their mutual performance in the context of seed quality have not been documented. Considering that germination is a critical stage for successful crop establishment, plant growth, yield potential, and resilience to stress, optimizing this phase through integrated biostimulant and fertilizer strategies represents a key step toward achieving more efficient and sustainable crop production systems. Understanding these interactions should advance the development of sustainable seed treatment strategies to stimulate seed germination and enhance seedling growth by simultaneously aiding microbial activity and seed metabolic functions. It was assumed that applying PGPR together with a nutrient complex will synergistically enhance soybean seed germination and early seedling development, outperforming the effects of *Br. japonicum* alone by improving nutrient uptake efficiency and activating key biochemical processes critical for germination and seedling vigor. Therefore, the aim of this research was to evaluate the effects of PGPR-based biostimulants, i.e., *Br. japonicum* alone or *Br. japonicum* with *A. chroococcum* and *B. subtilis*/*B. megaterium*, with a potentially complementary nutrient complex on seed germination and the initial seedling growth of two soybean cultivars under optimal laboratory conditions.

2. Materials and Methods

2.1. Plant Material

Seeds of the soybean cultivars 'NS Apolo' and 'NS Rubin' were obtained from the Legume Department, Institute of Field and Vegetable Crops, Novi Sad, Serbia (IFVCNS). The 'NS Apolo' is a medium variety that belongs to maturity group I. The plants have medium height and grey pubescent, while their grains are of medium size with a yellow seed coat and yellow hilum. The 'NS Rubin' is a late variety that belongs to maturity group II. Plants are medium height with brown pubescent, and have medium-sized grains with a yellow seed coat and yellow hilum.

2.2. Bacterial Strains

Bacterial strains were obtained from the culture collection of the Section for Microbiological Preparations of IFVCNS. *Bradyrhizobium* strains involved six strains of *Br. japonicum* (BJ1, BJ2, BJ4, BJ6, BJ7, and BJ8), primarily isolated from soybean root nodules collected from different locations in Vojvodina province [21]. These strains were selected based on their prominent PGP activities as well as their ability to enhance soybean nodulation in soils of different fertility [22]. *Bacillus* spp. were originally isolated from rhizosphere soil samples collected from different hosts and locations in Serbia. They involved four different *B. subtilis* (B5, B7, B13, and B32) and *B. megaterium* (B8, B12, B15, and B17) strains, selected on the basis of their PGP properties and positive effect on soybean seed germination and initial seedling growth [18,23]. *Azotobacter* strains involved three of the most effective PGP strains of *A. chroococcum* (A3, A6, A12), isolated from maize rhizosphere soil collected at the Rimski Šančevi experimental field of IFVCNS [24]. All bacterial strains used in this research are identified using 16S rDNA sequencing and deposited in the Nucleotide GenBank within the National Center for Biotechnology Information (NCBI) upon receiving an accession number (Supplementary Table S1).

2.3. Preparation of PGPR Biostimulants and Nutrient Complex

Bacterial strains were cultured in their respective liquid media: yeast extract mannitol broth (YEMB) for *Br. japonicum* (BJ) strains, nutrient broth (NB) for *Bacillus subtilis* (BS) and *B. megaterium* (BM) strains, and Burk's nitrogen-free broth (NFB) for *A. chroococcum*

(AC) strains. All media or media ingredients were purchased from Hi Media Laboratories Pvt. Limited, Mumbai, India. Strains were grown on a horizontal shaker (Edmund Bühler SM-30 B, Bodelshausen, Germany) set to 28 ± 2 °C and 100 rpm for 72 h (BJ) or 48 h (BS, BM, AC). Each strain was cultivated separately and subsequently mixed in equal ratios to obtain the final BJ, BS, BM, and AC suspensions, which were adjusted to a concentration of 10^9 colony-forming units per mL (CFU/mL). Prior to mixing, a dual culture assay was performed on respective agar plates, which confirmed that the selected PGPR strains are compatible. A commercially available formulation, Wuxal® Microplant (Aglucone Fertilizers GmbH & Co. KG, Düsseldorf, Germany), typically employed for foliar nutrition of cereals, fruits, and vegetables during intensive growth, was utilized in this study. It represents a nutrient complex (NC) in an aqueous solution of the following chemical composition of (% m/m): S—5.2; Mg—3; Mn—1.5; Fe—1; Zn—1; Cu—0.5; B—0.3; Mo—0.01. This solution was added to the respective bacterial suspensions as described below.

2.4. Seed Treatments

Seeds were surface disinfected in 1% sodium hypochlorite (Sigma Aldrich, St. Louis, MO, USA), washed with sterile distilled water five times, and then dried back to their original weight. Five seed treatments were tested: BJ treatment (5 mL of BJ bacterial suspension/100 seeds); BJ + NC treatment (4.8 mL of BJ bacterial suspension + 0.2 mL of NC solution/100 seeds); BJ + BS + AC + NC treatment (1.6 mL of BJ bacterial suspension + 1.6 mL of BS bacterial suspension + 1.6 mL of AC bacterial suspension + 0.2 mL of NC solution/100 seeds); BJ + BM + AC + NC treatment (1.6 mL of BJ bacterial suspension + 1.6 mL of BM bacterial suspension+ 1.6 mL of AC bacterial suspension + 0.2 mL of NC solution/100 seeds). Before treating seeds, the effect of the nutrient complex on selected bacteria was tested, showing its positive effect on their survival in appropriate liquid media. Seeds were then soaked in the abovementioned formulations for 30 min, air-dried on sterile filter paper at room temperature, and immediately used for the germination assay. Non-treated seeds were soaked in the same amount of sterile tap water and used as the control. Preparation of formulations and their application on seeds were done in the Section for Microbiological Preparations of IFVCNS.

2.5. Germination Assay

The germination test was performed in the Laboratory for Seed Testing of IFVCNS in order to assess the effect of prepared formulations on soybean seed quality and performance. The experiment was set up as a two-factorial design: cultivar × treatment, in three replicates. One replicate consisted of 100 seeds sown in germination box (240 × 150 mm) on sterilized and optimally moistened sand. The experiment comprised a total of 30 boxes (2 cultivars × 5 treatments in 3 repetitions). Samples were placed into a germination chamber (Conviron CMP 4030, Winnipeg, MB, Canada), programmed for a day/night regime of 16/8 h and a temperature of 25 °C, which are considered optimal conditions for soybean according to the ISTA Rules [25].

2.5.1. Determination of Seed Germination Parameters

The germination energy (*GE*), i.e., first germination count, described as the percentage (%) of seeds in a sample that germinate within a certain period, was counted considering only the seedlings with well-formed essential structures, five days after sowing [25]. The final germination (*FG*), defined as percentage (%) of healthy and well-developed seedlings, and abnormal seedlings (*AS*), defined as percentage (%) of seedlings without potential for later development into adequate plants, were counted eight days after sowing [25].

2.5.2. Determination of Seedling Growth Parameters

In order to measure shoot length (*SL*) and root length (*RL*) (mm), samples consisting of 25 randomly chosen soybean seeds per replication were germinated using rolled filter paper in the germination chamber, at the same conditions as for the germination test. Subsequently, 10 normal seedlings per replicate were randomly selected for further measurements of the shoot and root length on the 5th and 8th day of germination assessment. Shoot elongation rate (*SER*) and root elongation rate (*RER*) were calculated using the following formulas [26]:

$$SER = (SLE - SLS)/(TE - TS) \quad (1)$$

$$RER = (RLE - RLS)/(TE - TS) \quad (2)$$

where

SLS, *RLS*—shoot and root length (mm) at the start (5th day) of a measurement period;
SLE, *RLE*—shoot and root length (mm) at the end (8th day) of a measurement period;
$TE - TS$—time duration (days) between two measurement periods.

2.5.3. Determination of Seedling Biomass Accumulation

The fresh shoot weight (*FSW*) and fresh root weight (*FRW*) (g) of 10 seedlings were measured using analytical balance (Kern 770-13, KERN & Sohn GmbH, Balingen, Germany) on the 8th day. Afterwards, seedlings were oven dried at 80 °C for 24 h for determination of dry shoot weight (*DSW*) and dry root weight (*DRW*) (g).

2.5.4. Determination of Seedling Vigor Index

The seedling vigor index was calculated according to Abdul-Baki and Anderson [27], using the following formula:

$$SVI = (SL + RL) \times FG\ (\%) \quad (3)$$

where

SL, *RL*—shoot and root length (cm) on the 8th day;
FG—final germination (%) on the 8th day.
All determinations were performed in three replications.

2.6. Statistical Analysis

Five treatments were arranged in a complete randomized design (CRD) for laboratory experiment, with three replications. The data obtained were processed statistically using ANOVA and mean separation by Duncan's multiple range test (DMRT) ($p \leq 0.05$). The data were analyzed statistically using the STATISTICA 10.0 software (StatSoft Inc., Tulsa, OK, USA). Relationships between parameters was determined by Pearson's correlation analysis using R 4.2.2 software (R Foundation for Statistical Computing, Vienna, Austria). Principal component analysis (PCA) was performed based on comparing the effect of treatments using the freeware Minitab 17.1 software (Minitab Inc., State College, PA, USA).

3. Results

In the present study, the germination test was employed to characterize seed quality in relation to applied PGPR + NC formulations under optimal conditions in order to forecast the potential field emergence of treated soybean seeds. Table 1 presents the effect of cultivar, treatment and their interaction on soybean seed germination and the initial seedling growth. Factor *cultivar* (C) significantly affected germination energy, seedling vigor index, root

length and elongation rate, fresh and dry shoot weight, and dry root weight ($p \leq 0.001$), as well as shoot length and elongation rate ($p \leq 0.05$). Factor *treatment* (T) had a significant effect on all soybean parameters (abnormal seedlings, $p \leq 0.05$; other parameters, $p \leq 0.001$). Moreover, *cultivar* × *treatment* (C × T) interaction had a significant effect on all investigated parameters (root elongation rate, fresh shoot and root weight, $p \leq 0.01$; other parameters, $p \leq 0.001$), except abnormal seedlings.

Table 1. Analysis of variance (ANOVA) for parameters of two soybean cultivars after applying PGPR and nutrient complex as seed treatments.

Traits	Factor		
	Cultivar (C)	**Treatment (T)**	**C × T**
Germination Energy	***	***	***
Final Germination	ns	***	***
Abnormal Seedlings	ns	*	ns
Seedling Vigor Index	***	***	***
Shoot Length	*	***	***
Root Length	***	***	***
Shoot Elongation Rate	*	***	***
Root Elongation Rate	***	***	**
Fresh Shoot Weight	***	***	**
Fresh Root Weight	ns	***	**
Dry Shoot Weight	***	***	***
Dry Root Weight	***	***	***

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns—not significant.

The influence of seed treatments with PGPR and nutrient complex on germination parameters and seedling vigor index is presented in Table 2. A significant effect of all treatments was observed in germination energy in relation to the control. On average for both soybean cultivars, the increase in germination energy over control ranged from 6.7% to 9.5%, and the most favorable effect was achieved with PGPR consortia supplemented with nutrient complex (BJ + AC + BM + NC and BJ + AC + BS + NC), which also had a significantly higher effect compared to treatments consisting of *Br. japonicum* alone (BJ) or with nutrients (BJ + NC). Also, the effect of seed treatments on germination energy was higher in 'NS Rubin' (5.3–15%) in comparison with 'NS Apolo' (3.7–8%). Regarding the final germination, an increase over control was obtained in all treatments, in a range of 3.7–16% ('NS Apolo') and 1–12.3% ('NS Rubin'), i.e., 2.3–10.8% (cultivar average). On average for both cultivars, all PGPR treatments with nutrient complex induced a significantly higher final germination as compared to both control and individual application of *Br. japonicum* (BJ). However, there were no significant differences among the bacterial treatments with nutrients for final germination. Although a positive impact of all PGPR + NC formulations was noted, only the BJ + AC + BM + NC treatment significantly reduced the number of abnormal seedlings compared to the control and *Br. japonicum* single usage, as observed by the cultivar average. The same treatment had the best effect in tested cultivars, leading to the reduction of −2% ('NS Apolo') and −2.7% ('NS Rubin', $p \leq 0.05$) in relation to the control. Moreover, all applied seed treatments significantly improved seedling vigor index in relation to the control, leading to an average increase of 15.7–34.2%, with a higher effect in 'NS Rubin' (19.0–47.8%) compared to 'NS Apolo' (12.9–38.2%). On average, the best effect was achieved in PGPR treatments with nutrient amendment, in the following order: BJ + AC + BM + NC > BJ + NC > BJ + AC +BS + NC.

Table 2. Effect of seed treatments on soybean seed germination parameters and seedling vigor index.

Treatments	Germination Energy (%)	Final Germination (%)	Abnormal Seedlings (%)	Seedling Vigor Index
		'NS Apolo'		
Control	57.7 g	77.7 ce	3.33 ab	1923 c
BJ	64.3 ef	81.3 bcd	3.67 ab	2172 b
BJ + NC	65.7 de	93.7 a	4.00 a	2658 a
BJ + AC + BS + NC	61.3 f	81.3 bcd	2.33 ab	2199 b
BJ + AC + BM + NC	65.0 e	82.7 bc	1.33 b	2360 b
		'NS Rubin'		
Control	63.3 ef	77.3 e	4.00 a	1619 d
BJ	70.3 c	78.3 bcd	3.67 ab	1927 c
BJ + NC	68.7 cd	83.0 b	1.67 ab	2037 c
BJ + AC + BS + NC	78.3 a	89.3 a	1.67 ab	2264 ab
BJ + AC + BM + NC	75.0 b	89.7 a	1.33 b	2392 b
		Average		
Control	60.5 c	77.5 b	3.67 a	1771 d
BJ	67.3 b	79.8 b	3.67 a	2049 c
BJ + NC	67.2 b	88.3 a	2.83 ab	2347 a
BJ + AC + BS + NC	69.8 a	85.3 a	2.00 ab	2232 b
BJ + AC + BM + NC	70.0 a	86.2 a	1.33 b	2376 a

Data are presented as means (n = 3). Differences between treatments were analyzed using the Duncan's multiple range test ($p \leq 0.05$). Means within each trait followed by the same letters are not significantly different. Note: BJ—*Bradyrhizobium japonicum*; BS—*Bacillus subtilis*; BM—*Bacillus megaterium;* AC—*Azotobacter chroococcum*; NC—nutrient complex.

Additionally, the examined seed treatments also had a positive effect on initial plant growth (Table 3), resulting in significant increases in the average values of the tested parameters compared to the control: shoot length (8.8–15.8%), root length (13.9–25.5%), shoot elongation rate (10.4–33.2%), and root elongation rate (27.4–68.2%). On average, the highest effect on the abovementioned parameters of tested soybean cultivars was obtained after applying PGPR consortia and nutrients (BJ + AC + BM + NC). In 'NS Apolo', shoot length was increased by 2.6–6.5% as compared to the control, while in 'NS Rubin' the increase was higher and ranged from 14.9 to 30.4%, while in the case of root length, the cultivars responded quite similarly to treatments with increases of 11.7–26.4% and 13.5–24.5%, respectively. The treatment effect was most noticeable in the case of root elongation rate, where the obtained increase in 'NS Apolo' and 'NS Rubin' ranged from 28.2% and 7.5% to 67.4% and 69.2%, respectively. Similar results were recorded for shoot elongation rate, but only in 'NS Rubin' (22.8–63.8% increase over control), whereas the effect of applied treatments on this growth parameter in 'NS Apolo' was not significant (1.4–10.9% increase over control).

Furthermore, all seed treatments significantly improved the accumulation of soybean biomass in relation to the control (Table 4). On average, the percentage increases over control were in the following range: fresh shoot weight (8.9–15%); fresh root weight (11.3–16.3%); dry shoot weight (5.3–12.3%); and dry root weight (16.8–24.8%). In 'NS Apolo', fresh and dry biomass was increased by 5.3–15.3% and 3.8–13.7% (shoot parameters) and by 12.3–15.7% and 13.0–29.6% (root parameters), respectively. In 'NS Rubin', fresh and dry weight was enhanced by 12.2–17.0% and 5.3–15.5% (shoot parameters) and by 8.9–20.2% and 17.9–23.1% (root parameters). The highest effect on fresh shoot weight was observed in BJ, BJ + NC, and BJ + AC + BS + NC treatments, without statistically significant differences between them. Similarly, the highest values of fresh root weight were recorded after applying *Br. japonicum* (BJ), followed by PGPR consortium (BJ + AC + BS + NC). Interestingly, the use of *Br. japonicum* alone had the best effect on dry shoot weight, while

BJ + AC + BS + NC and BJ + NC treatments stood out in terms of their efficacy on dry root weight.

Table 3. Effect of seed treatments on soybean seedling growth parameters.

Treatments	Shoot Length (cm)	Root Length (cm)	Shoot Elongation Rate	Root Elongation Rate
		'NS Apolo'		
Control	120 de	128 cd	20.4 bcd	15.2 c
BJ	124 cd	143 b	20.7 bcd	19.8 b
BJ + NC	123 cd	161 a	20.4 bcd	22.5 ab
BJ + AC + BS + NC	127 bc	143 b	21.2 bc	19.4 b
BJ + AC + BM + NC	124 cd	162 a	22.6 ab	25.4 a
		'NS Rubin'		
Control	102 f	107 e	14.9 e	12.6 c
BJ	117 e	129 cd	18.3 d	19.5 b
BJ + NC	124 cd	122 d	19.1 cd	13.6 c
BJ + AC + BS + NC	129 b	125 d	22.3 ab	15.9 c
BJ + AC + BM + NC	133 a	134. c	24.4 a	21.3 b
		Average		
Control	111 c	118 d	17.6 d	13.9 c
BJ	121 b	136 c	19.5 c	19.6 b
BJ + NC	123 b	142 b	19.8 c	18.0 b
BJ + AC + BS + NC	128 a	134 c	21.7 b	17.7 b
BJ + AC + BM + NC	128 a	148 a	23.5 a	23.4 a

Data are presented as means (n = 3). Differences between treatments were analyzed using the Duncan's multiple range test ($p \leq 0.05$). Means within each trait followed by the same letters are not significantly different. Note: BJ—*Bradyrhizobium japonicum*; BS—*Bacillus subtilis*; BM—*Bacillus megaterium;* AC—*Azotobacter chroococcum*; NC—nutrient complex.

Table 4. Effect of seed treatments on soybean seedling biomass accumulation.

Treatments	Fresh Shoot Weight (g)	Fresh Root Weight (g)	Dry Shoot Weight (g)	Dry Root Weight (g)
		'NS Apolo'		
Control	8.674 f	1.587 c	1.128 h	0.115 h
BJ	10.003 c	1.782 b	1.282 f	0.130 g
BJ + NC	9.498 de	1.807 b	1.180 g	0.149 d
BJ + AC + BS + NC	9.304 e	1.836 b	1.187 g	0.147 d
BJ + AC + BM + NC	9.133 e	1.807 b	1.171 g	0.139 e
		'NS Rubin'		
Control	9.705 cd	1.621 c	1.483 e	0.134 f
BJ	11.144 ab	1.949 a	1.651 b	0.163 ab
BJ + NC	11.316 a	1.816 b	1.585 c	0.159 bc
BJ + AC + BS + NC	11.358 a	1.818 b	1.561 d	0.165 a
BJ + AC + BM + NC	10.886 b	1.766 b	1.713 a	0.158 c
		Average		
Control	9.190 c	1.604 c	1.305 d	0.125 c
BJ	10.573 a	1.866 a	1.466 a	0.146 b
BJ + NC	10.407 a	1.811 b	1.382 c	0.154 a
BJ + AC + BS + NC	10.331 a	1.827 ab	1.374 c	0.156 a
BJ + AC + BM + NC	10.009 b	1.786 b	1.442 b	0.148 b

Data are presented as means (n = 3). Differences between treatments were analyzed using the Duncan's multiple range test ($p \leq 0.05$). Means within each trait followed by the same letters are not significantly different. Note: BJ—*Bradyrhizobium japonicum*; BS—*Bacillus subtilis*; BM—*Bacillus megaterium*; AC—*Azotobacter chroococcum*; NC—nutrient complex.

Positive relationships between most investigated parameters confirm the beneficial effects of applied treatments on seed germination and early growth of examined soybean cultivars in optimal laboratory conditions (Figure 1). A significant positive dependence was noticed between germination energy and fresh shoot weight, dry shoot weight, and dry root weight; final germination and seedling vigor index; shoot length and shoot elongation rate, and seedling vigor index; root length and root elongation rate, and seedling vigor index; dry root weight and fresh root weight, and dry shoot weight; dry shoot weight and shoot and root elongation rate; seedling vigor index and shoot and root elongation rate. Negative relations were observed between abnormal seedlings and other examined parameters; root length and germination energy, fresh shoot weight, dry shoot weight, and dry root weight; fresh shoot weight and root elongation rate; dry shoot weight and shoot elongation rate, root elongation rate, and seedling vigor index. However, a significant negative dependence was recorded only between abnormal seedlings and shoot length and elongation rate.

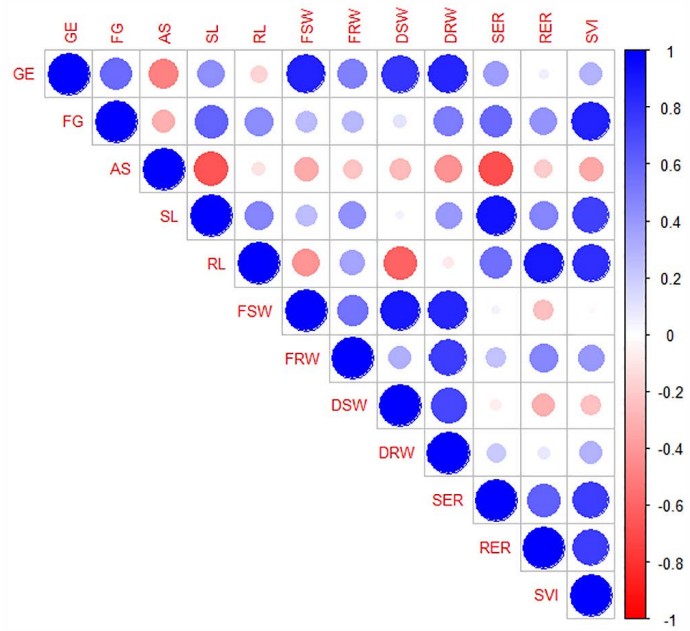

Figure 1. Correlation heat map of seed germination and initial seedling development of soybean in optimal laboratory conditions. Blue colors indicate positive relationships, and red colors indicate negative relationships. Note: (GE) germination energy; (FG) final germination; (AS) abnormal seedlings; (SL) shoot length; (RL) root length; (FSW) fresh shoot weight; (FRW) fresh root weight; (DSW) dry shoot weight; (DRW) dry root weight; (SER) shoot elongation rate; (RER) root elongation rate; (SVI) seedling vigor index.

Moreover, the association between applied seed treatments and examined cultivars is displayed via the PCA biplot (Figure 2). The two soybean varieties, 'NS Apolo' (maturity group I) and 'NS Rubin' (maturity group II), were clearly separated by the PCA, suggesting inherent genetic or phenotypic differences. In both cultivars, the formulations were clearly separated from their respective controls due to their promoting effect on the examined soybean parameters. Furthermore, clustering patterns across the several treatment groups were noted within each variety, indicating that the treatments significantly affected. For example, especially in 'NS Rubin', the treatment that included BJ + BM + AC + NC combination clearly distinguished from other groups. This treatment was followed by bacterial consortium composed of BJ + BS + AC + NC that was also clearly separated from BJ and BJ + NC. In 'NS Apolo', the treatments BJ + BM + AC + NC and BJ + NC on one side, as well as the treatments BJ and BJ + BS + AC + NC on the other, were grouped

due to their similar effect, with the former group having an advantage over the latter. These findings show that the total variability shown in the dataset is influenced by both treatment effects and differences on variety level. The robustness of the observed patterns was confirmed by the fact that the first two principal components explained 92.5% of the variation (PC1: 54.19%, PC2: 38.31%).

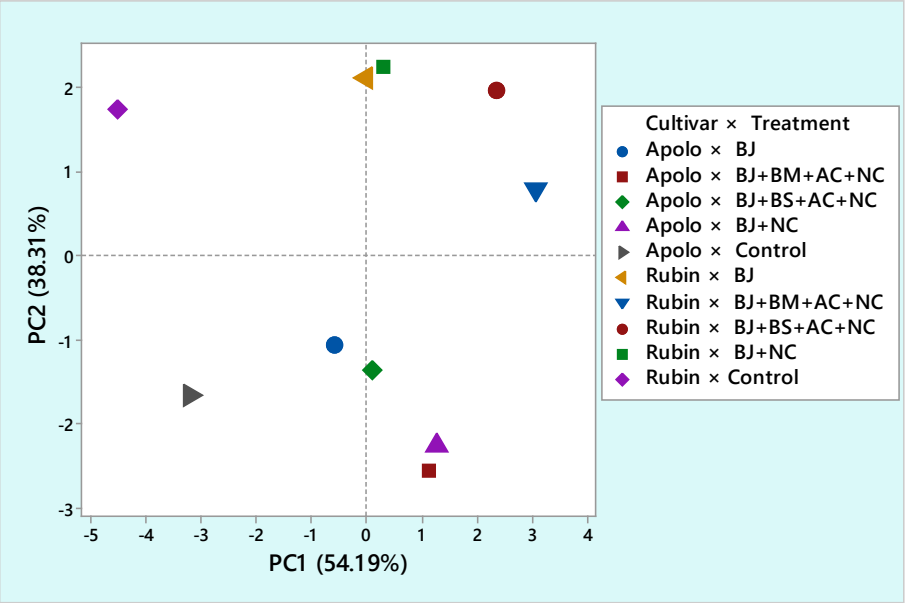

Figure 2. Principal component analysis (PCA) for the effect of seed treatments on the examined parameters of soybean cultivars in germination test.

4. Discussion

This research focuses on the effect of novel formulations based on PGPR biostimulants and nutrients and their synergy, providing compelling evidence that applied seed treatments significantly promoted soybean seed germination and seedling early growth. Beneficial effects are probably attributable to the complementary mechanisms derived from both the bacterial and nutritional components of the formulations.

PGPR strains such as *Bradyrhizobium*, *Azotobacter*, and *Bacillus* are well-known for their multifunctional roles in promoting plant growth. In the context of this research and the obtained improvements in germination and seedling parameters, the most important mechanism of the applied bacteria is phytohormone production, including indole-3-acetic acid (IAA), gibberellins, and cytokinins, which can directly stimulate cell division, elongation, and differentiation [28,29]. These hormones play pivotal roles in regulating key developmental processes, including the release of seed dormancy (via gibberellins), initiation of root and shoot development (via IAA), and modulation of the root-to-shoot ratio and nutrient translocation (via cytokinins) [30,31]. When NC are co-applied, they provide vital macro- and micronutrients that function as cofactors in enzymatic reactions involved in hormone biosynthesis and signal transduction, thereby enhancing the growth-promoting effects initiated by PGPR [32]. It has been reported that seed treatments with IAA-producing PGPR improve germination, root growth, architecture, and biomass, while the development of a more robust root system and consequent enhancement of nutrient uptake led to the increase in overall plant growth and yield [33–36]. For instance, seed coating with bacteria that produce high levels of IAA significantly enhanced germination, mean germination time, shoot and root length, and accumulation of N, P, K, and Ca in the seedlings under laboratory and greenhouse conditions [36]. Simultaneously, NC contributes to the enrichment of the rhizosphere with readily available nutrients and

promotes proliferation of beneficial microorganisms in this zone [37]. This dual stimulation enhances root nutrient uptake efficiency, promotes greater water absorption, and leads to improved root anchorage and overall seedling vigor [38]. These mechanisms are consistent with our findings, where the combined application of PGPR and NC resulted in the most pronounced improvements in root and shoot elongation rates, as well as in the seedling vigor index. Seed germination is characterized by the rapid mobilization of stored reserves, activation of respiratory pathways, and initiation of protein synthesis. PGPR can facilitate and accelerate these processes by producing gibberellins that stimulate the activity of hydrolytic enzymes, such as α-amylase, which degrade starch into readily usable sugars [39]. Nutrient complexes supply readily accessible ions and essential cofactors, including Mg^{2+}, Zn^{2+}, and Fe^{2+}, which play a critical role in supporting metabolic functions by facilitating enzymatic activity and promoting protein biosynthesis [12–15].

Simultaneously, PGPR makes nutrients more available to the emerging seedlings through mechanisms such as phosphate solubilization, nitrogen fixation, siderophore production, and improved root system architecture [9,40]. Plants benefit from improved nutrient availability and uptake efficiency, which collectively minimize nutrient losses and enhance physiological nutrient utilization [40,41]. During the early stages of development, seedlings are highly susceptible to various abiotic stresses such as drought, salinity, and oxidative damage. PGPR can confer systemic stress tolerance by upregulating the activity of antioxidant enzymes (e.g., superoxide dismutase, catalase, and peroxidase), promoting the accumulation of osmoprotectants like proline, and modulating the levels of stress-related phytohormones, particularly through the suppression of ethylene production [42]. Micronutrients such as Zn, Fe, Cu, Mg, and Mo are crucial for maintaining cellular membrane stability and supporting the antioxidant defense system [43]. When included in the nutrient complex, these elements enhance the plant's stress resilience, thereby complementing and reinforcing the protective effects mediated by PGPR [42]. Furthermore, *Bacillus* strains protect seeds from pathogens through antimicrobial compounds and endospore formation, enabling survival in conditions harmful to nitrogen-fixing bacteria [44–46]. The advantage of their combined application in soybean is reflected especially in the early stages of plant growth, i.e., before nodulation, when nitrogen fixed in soil and other bioactive compounds produced by PGPR lead to higher germination, stronger seedlings, better root establishment and later symbiotic nitrogen fixation. This ultimately contributes to yield formation starting from the initial stages of plant growth and development [47]. Thus, Kozieł et al. [48] found that pre-sowing seed treatments with *Br. japonicum* alone or with *A. chroococcum* were equally efficient in improving soybean nodulation and yield in comparison to the control. Furthermore, co-inoculation of soybean with *Br. diazoeffciens* and *Bacillus velezensis* enhanced nitrogen fixation efficacy as manifested by the formation of bigger nodules, stimulated by IAA, cytokinin, and other substances secreted from the *Bacillus* strain [49]. Moreover, the effectiveness of complex soybean seed inoculation with *Br. japonicum* and *Bacillus* sp. is reflected on rhizosphere microbiota and yield attributes, with a simultaneous increase in soybean yield and seed protein content [50]. PGPR consortia tend to be more effective than single inoculants, which are confirmed by this research. Such biostimulants are also more adaptable and resilient to varying environmental conditions, which is an important consideration for successful lab-to-field transition. It was observed that PGPR-based inoculants composed of different species and/or strains with multiple beneficial functions could shape the rhizosphere microbial communities and improve soybean growth [51].

In parallel, the nutrients have an essential role in maintaining enzyme activities, energy transfer, and general metabolic activity during germination and seedling growth, considering that seed formation requires an appropriate nutrient load from the seed coat to

the filial endosperm [52]. Besides their crucial role in accelerating early metabolic activation and cellular expansion in treated seeds, macro- and micronutrients are essential for diverse metabolic processes and functions of PGPR. The presence of the nutrients presumably complemented PGPR activities by improving nutrient uptake as well as physiological efficiency in the emerging seedlings. When combined as a seed treatment, PGPR-nutrient formulations form a synergistic cascade, boosting bacterial colonization, phytohormone production, stress protection, and nutrient acquisition that consequently improve soybean seed germination and early seedling development [17,20]. The combined use of PGPR and NC in our study clearly enhanced physiological and metabolic processes during these critical phases, resulting in significant improvements in seedling vigor, as demonstrated by higher germination percentage, increased root and shoot length, and greater fresh and dry biomass. For instance, an observed increase of 34.2% in seedling vigor index exceeds the highest improvement reported in a previous study, including a 12.5% increase resulting from *B. megaterium* alone applied as a biopriming agent for soybean seeds [18]. These results suggest that the nutrient complex and PGPR consortia contribute significantly to amplifying the beneficial effects of applied seed treatments. Such metrics are vital since seedling vigor strongly influences crop establishment success, weed competitiveness, and tolerance to early environmental stresses. Vigorous seedlings usually develop more efficient root systems, leading to improved nutrient and water absorption, which contributes to greater stand uniformity and ultimately enhances yield potential.

Both cultivars positively responded to applied formulations, indicating their overall efficiency and potential for wider application. However, 'NS Rubin' better responded to seed treatments, with higher value increases in investigated parameters and more noticeable differences in the effect of individual treatments. This is in agreement with previous studies that also showed genotype-specific interactions with the applied seed treatments in soybean [18,53]. Different soybean cultivars exhibit varying feedbacks, most likely because of genetic differences in root exudation profiles as well as their differential ability to interact with the bacteria and nutrients, harnessing the benefits they provide. Kuzmicheva et al. [54] proposed that variety-dependent interactions of soybean with PGPR can be related to root exudation rate and exudate composition and different abilities of PGPR to metabolize and transform such exudates. In addition to root exudation profiles, Egamberdieva et al. [55] reported that seed coat properties and inherent metabolic capabilities may influence the extent of cultivar responsiveness to PGPR and nutrients, influencing their entry into the seed or processing the inputs in varying ways. The results of this study offer important perspectives for the development of next-generation biofertilizer formulations. Conventional biofertilizers typically depend solely on PGPR strains, which can exhibit variable effectiveness in the field because of nutrient limitations or environmental stresses [56]. Our results indicate that combining PGPR with suitable nutrient complexes leads to stronger and more consistent plant responses, especially during key early stages of growth. This combined strategy supports the objectives of sustainable agriculture by minimizing reliance on synthetic chemicals, improving nutrient uptake efficiency through biological means, and promoting the creation of multifunctional bio-inputs that deliver both microbial and nutritional advantages [57]. The beneficial effects of combined PGPR-nutrient treatments highlight their intrinsic contribution in promoting early developmental processes under optimal laboratory conditions, where environmental stress is minimized. However, it is important to acknowledge that the effectiveness of such treatments in field conditions may be affected by multiple biotic and abiotic factors. Future research should pay attention to the formulation stability and long-term effects of promising treatments on plant performance across different cultivars and growth stages during field evaluations, as well as the underlying molecular and physiological mechanisms behind their beneficial actions.

5. Conclusions

The present study represents the first experimental confirmation of the integrated use of PGPR consortia with a nutrient complex as novel formulations intended for soybean seed treatments, showing their great potential for improving the germination and the initial growth under optimal laboratory conditions. Overall, the treatment effect was significantly positive or demonstrated a positive trend in relation to the control, suggesting that synergy of PGPR and nutrients contributed to physiological responses during the initial stages of plant development. The notable differences between cultivars and how they interact with treatments suggest that choosing the right treatment for each variety may have an extra effect on promoting seed quality and performance. The most pronounced effect of seed treatments was on the root elongation rate, seedling vigor index, and shoot elongation rate, followed by other parameters of root, shoot, and seed germination of both cultivars. Also, a specific influence of individual treatments was observed depending on the examined soybean parameters, such as the advantage of the BJ + BM + AC + NC treatment in terms of seed germination and early seedling growth, while the BJ and the BJ + BS + AC + NC treatments were among the most efficient when it comes to seedling biomass accumulation. These formulations, particularly PGPR consortia combined with a nutrient complex (BJ + BM + AC + NC), demonstrate potential for future application and can be recommended for seed treatment prior to field sowing as a promising approach to improve crop emergence and establishment. The synergistic interaction between PGPR and the nutrient complex not only enhances early plant development but also contributes to a more sustainable and efficient agricultural system by improving nutrient use efficiency, reducing dependence on synthetic inputs, and promoting soil health. Given the observed results, this approach may be recommended for other leguminous crops as well. Further field trials are required to validate the benefits of these combined formulations as effective, consistent, and crop-specific tools across different legume species and environmental conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture15192022/s1, Table S1: Bacterial strains used for the preparation of PGPR formulations.

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