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Effectiveness of Co-inoculation with *Pseudomonas koreensis* and Rhizobia on Growth, Nodulation and Yield of Common Bean (*Phaseolus vulgaris* L.)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The establishment of nodulation, nitrogen fixation and productivity in legumes is affected by specificity of inoculant's strain and effectiveness as well as interactions with rhizosphere microorganisms. This study aimed to isolate and identify *Pseudomonas* isolates and investigates the effect of *Rhizobium* - *Pseudomonas* co-inoculation on growth, nodulation and yield of common bean (*Phaseolus vulgaris* L.) cv. Nebraska under greenhouse and lyzemeter conditions. In total, 7 *Pseudomonas* isolates were isolated from clay soil and evaluated as co-inoculation by measuring the symbiotic N_2-fixation parameters under greenhouse. The nine treatments consisted of (seven *Pseudomonas* isolates (PS1-PS7) combinations with *R. leguminosarium sembiovar phaseoli* (TAL-3612), one inoculated with *R. leguminosarium sembiovar phaseoli* (TAL-3612), and one control). One isolate (PS7) exhibited the highest values of growth, symbiosis, photosynthetic and nitrogen content. Based on 16S rRNA sequence, this strain was shown to belong to *Pseudomonas koreensis*.

Lyzemeter experiment was carried out as complete randomized block designed which consisted of four treatments; T_1= Control, T_2= Inoculation with *R. leguminosarium sembiovar phaseoli* (TAL-

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3612), T_3= Inoculation with *P. koreensis,* and T_4= T_2+T_3 with 5 replicates. Co-inoculation treatment significantly enhanced plant height, nodulation, dry biomass and increased macroelements content (N, P and K) and microelements content (Zn, Mn, Fe and Cu) over single inoculation and control at 30 and 60 days of sowing. The treatments T_4 and T_3 recorded the highest values at all growth stages, they recorded 241.00 and 225.66 µg TPF g^{-1} soil day^{-1} at 30 days and 249.33 and 235.00 µg TPF g^{-1} soil day^{-1} at 60 days and 203.66 and 179.66 µg TPF g^{-1} soil day^{-1} at harvest for dehydrogenase activity. Also, phosphatase activity in soil generally reduced with the aging of the plant. Similar trend are showed for seed yield and quality assessment.
Therefore, co-inoculation with *R. leguminosarium sembiovar phaseoli* and *P. koreensis* could be an effective biofertilization for common bean plant production.

Keywords: Co-inoculation; common bean; Rhizobium; Pseudomonas; seed yield.

1. INTRODUCTION

In natural and agricultural systems, bulk soil is often resource-limited and cause major constraints to the growth of microorganisms [1]. On the other hand, highly diverse environment occur in the rhizosphere between plant roots, microbes and the soil [2]. In modern agricultural practices, millions of tons of different fertilizers are indiscriminately used to achieve optimum crop yields. These fertilizers leach into the grounds and disrupt the composition and functions of beneficial rhizosphere microorganism and indirectly human health. So, due to the alarmingly very high costs of fertilizers and some environmental hazards [3].

Common bean (*Phaseolus vulgaris* L.) is one of the most important crops and represents 50% of the grain legumes consumed worldwide. It is considered as a nearly perfect food mainly because of its high protein content (15%), fiber, carbohydrates, and 30% of the caloric requirement to the world's population as well as other daily food needs such as vitamins and minerals Cu, Ca, Fe, Mg, Mn, Zn [4,5]. In several studies [5-12], who reported that common bean is usually considered a poor nitrogen-fixing legume, poor nodulation and variable response to inoculation is mainly attributed to intrinsic characteristics of the host plant as well as the great sensitivity to other nodulation limiting factors, such as high rates of N fertilizer used, high temperatures and soil dryness.

In agricultural practices, about 80% of Biological Nitrogen Fixation (BNF) come from symbioses formed between leguminous plants and species of *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium*, and *Allorhizobium*, and absorbed directly by the plants and so is less susceptible to volatilization, denitrification, and leaching as well as improve organic fertility and farming system flexibility [13, 14]. In this context, the discovery of rhizobia in 1886, has provided some relief to the poor agronomic practitioners largely due to low cost and abundant availability [15,16]. Indeed, interactions between rhizobia with consortium of microorganisms and legumes plants have been identified for some time as essential in vegetative cover productivity and diversity [17,18], due to complementary phytostimulation traits might enhance legume growth for example, IAA, cytokinin and gibberellin, solubilizing non available P, synthesizing ACC deaminase, producing siderophores and increase nodulation more than any of the single-strain inoculations and secretion of B vitamins by the other microorganisms [19,20,21].

Pseudomonas spp. are a major component of the microbial flora, which live in close association with various types of agricultural crops. Their association with plant materials has been related to their ability to colonize and produce plant growth promoting compounds within the rhizosphere [22,23]. Also, *Pseudomonas* strains have been shown to increase plant growth and nutrient uptake of maize, wheat, and legumes in different soils and temperatures [12,24,25,26].

The new fashion in agricultural systems is the use of microbial consortiums of Plant Growth Promoting Bacteria (PGPB), including co-inoculation with rhizobia and other microorganisms. Therefore, the use co-inoculants can be affected by various factors such as the specificity of inoculant's strain and effectiveness, strain inherent potential and genotype, optimal inoculation dose, composition of root exudates, temperature variation as well as interaction of applied inocula with rhizospheric microflora [27,28]. Several studies in greenhouse and field showed the improvement of legume symbiosis and other microorganisms (PGPR),

due to enhance nodulation, nitrogen fixation and plant productivity in pea and alfalfa [29,30]; common bean and soybean [8]; *Medicago truncatula* [24]; Mungbean [31]; chickpea [32, 33]; Common bean [5,10,12]; Pea [25]; alfalfa [34]; Lucerne [35] and Soybean [11,36,37].

The aim of our study was to isolate and identify *Pseudomonas* isolates, as well as to investigate the effect of co-inoculation with specific rhizobia and the most effective *Pseudomonas* strain to improve nodulation, plant growth and yield of common bean seeds under greenhouse and lyzemeter conditions.

2. MATERIALS AND METHODS

2.1 Organism and Culture Conditions

2.1.1 *Pseudomonas*

Pseudomonas were isolated from clay soil using King's B (KB) agar medium [38], following serial dilution and plating technique. The plates were incubated at 30°C for 48 h. Colonies were picked up, purified by repeated streaking on the same medium and well isolated single colonies were transferred to KB slants to use in further studies. Bacterial identification of the isolates were confirmed by a series of morphological, physiological and biochemical tests as described by Bergey's Manual of Determinative Bacteriology [39].

2.1.2 *Rhizobium*

Rhizobium leguminosarium sembiovar phaseoli (TAL-3612), was provided from Bacteriology Laboratory, Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt. Pure cultures were routinely maintained on Yeast Extract Mannitol Agar (YEMA) medium [40].

2.2 Greenhouse Experiment

Leonard's jars were used *in vivo* to evaluate the effect of co-inoculation with different *Pseudomonas* isolates and *Rhizobium leguminosarium sembiovar phaseoli* (TAL-3612) to improve the growth, nodulation and nitrogen fixation of common bean (*phaseolus vulgaris* L.) cv. Nebraska under sterilized conditions.

A completely randomized design experiment comprised of 9 treatments (seven *Pseudomonas* isolates (PS1-PS7) combinations with *Rhizobium leguminosarium sembiovar phaseoli* (TAL-3612), one inoculated with *Rhizobium leguminosarium sembiovar phaseoli* (TAL-3612), and one control). Jars were filled with clean sand which had been treated with 0.1 M HCl and washed several times with distilled water and the Jars were autoclaved twice at 1.5 par, 121°C for 4 h. Seeds of common bean were surface-sterilized using standard methods [41]. Seeds were sown (2 seeds Jar^{-1}) and inoculated by pippeting 2 ml of 10^8 CFU ml^{-1} with different *Pseudomonas* isolates and 2 ml of 10^8 CFU ml^{-1} rhizobial liquid culture around each seed. Jars were irrigated twice weekly with nitrogen free nutrient solution prepared as described by [42].

After 45 days from sowing, plants were uprooted and evaluated for shoot length (cm), root depth (cm), shoot dry weight (g), root dry weight (g), number of nodules, nodules dry weight (g), total chlorophyll and N content (mg $plant^{-1}$).

2.3 Molecular Identification

The most effective *Pseudomonas* isolates to improve the growth, nodulation and nitrogen fixation of common bean were chosen to identify by Polymerase Chain Reaction (PCR) at Sigma Scientific Services Co., Giza, Egypt. Genomic DNA of the test bacterial isolates grown on KB broth was extracted with GeneJet Bacterial Genomic DNA Extraction Kit (Fermentas).

The 16S rRNA gene of the isolate was amplified by using universal primers Forward and Reverse (F, 5-AGA GTT TGA TCC TGG CTC AG-3 and R, 5- GGT TAC CTT GTT ACG ACT T-3) used to obtain a PCR product of ~ 1.5 kb. The sample was placed in a hybridthermal reactor thermocycler (Maxima Hot Start PCR Master Mix (Fermentas). The PCR products were analyzed on 1% (w/v) agarose gels and sent to GATC (Germany) for sequencing using ABI 3730xl DNA sequencer.

The resultant 16S rRNA gene sequences were compared to the GenBank database by using the National Center for Biotechnology Information database (NCBI), using BLASTN program. A dendrogram was constructed using the neighbour-joining method. Also, confidence in tree topology was determined.

2.4 Lyzemeter Experiment

Lyzemeter experiment was carried out during summer season of 2017 in Sakha Agricultural Research Station, Kafr El-Sheikh Governorate,

Egypt. The study aimed to clarify the impact of co-inoculation with *Pseudomonas koreensis* MG209738 with *Rhizobium leguminosarium sembiovar phaseoli* (TAL-3612) on growth, nodulation and yield of common bean (*phaseolus vulgaris* L.) cv. Nebraska.

The Lyzemeter experiment was conducted in clayey soil in texture having the following characteristics: pH, 7.10; EC, 3.36 dSm^{-1}; organic matter (%), 1.53; particle size distribution sand, silt and clay (%), 23.3, 22.1 and 54.6, respectively; soluble cations Ca^{+2}, Mg^{+2}, Na^{+} and K^{+} (meq L^{-1}), 4.38, 8.18, 9.24 and 0.20, respectively; soluble anions CO_3^{--}, HCO_3, Cl^{-} and SO_4^{--} (meq L^{-1}), 0.0, 3.75, 6.72 and 11.53, respectively; available N (mg Kg^{-1}), 35.1; available P (mg Kg^{-1}), 8.10; available K (mg Kg^{-1}), 250.7; Also, total count of bacteria, 210 x 10^6 CFU g^{-1}; total count of fungi, 81 x 10^4 CFU g^{-1} and total count of actinomycetes, 64 x 10^5 CFU g^{-1}. Physical, chemical and biological properties were determined according to the standard methods reported by [42,43,44], respectively.

The experiment undertaken in lyzemeter composed of 20 units each of 80 × 80 cm, and it was carried out as 4 x 5 complete randomized block designed. It comprised 4 inoculation treatments; T_1= Control, T_2= Inoculation with *Rhizobium leguminosarium sembiovar phaseoli* (TAL-3612), T_3= Inoculation with *Pseudomonas koreensis* MG209738, T_4= T_2+T_3, with 5 replicates for each treatment. Common bean seeds were planted at the rate of 4 seeds per hole with 20 cm space. Thinning was done after complete germination, leaving two plant per hole.

The inoculation treatments were prepared as peat-based inoculums, 15 ml of 10^8 CFU ml^{-1} from each culture per 30 g of sterilized carrier and mixed with the seeds before sowing using a sticking material. Also, ammonium sulphate (20.5% N) was the N source which, added the active fertilizer dose for all treatment before sowing, and two equal doses at 30 and 45 days from seed sowing for T_1 and T_3 treatments. The other recommended agricultural practices were used such as calcium superphosphate (15% P_2O_5) and potassium sulphate (48% K_2O).

2.5 Growth Parameters

At 30 and 60 days after sowing, plant samples were taken to determine plant height (cm), number of nodules $plant^{-1}$, dry weight of plant (g $plant^{-1}$), dry weight of nodules (g $plant^{-1}$), while number of pods, pod yield (g $plant^{-1}$), seed yield (ton ha^{-1}), total carbohydrate (%) and protein (%) of common bean plants were determined at harvest.

2.6 Plant Mineral Content

Nitrogen, phosphorus and potassium content (mg $plant^{-1}$) were determined according to the methods described by [43]. On the other hand, micronutrients content (mg $plant^{-1}$), Zn, Mn, Fe and Cu were measured using atomic adsorption spectrophotometer (Perkin Elmer 3300) according to [45].

Total carbohydrate contents were extracted from dry finely ground common bean seeds (powdered), according to [46] and estimated colourimetrically by the phenol-sulphuric acid method as described by [47]. For protein content of seeds was calculated as total N × 6.25 [48]. On the other hand, enzyme activity of dehydrogenase and phosphatase was recorded at 30, 60 and harvest according to [49,50], respectively.

2.7 Statistical Analysis

Data obtained were subjected to the analysis of variance and treatment means were compared using the L.S.D methods and the difference among treatment means was compared by high range statistical domain (HSD) using Tukey test at 5 % probability according to [51].

3. RESULTS AND DISCUSSION

3.1 Identification and Characterization

In the present study, a total of 7 *Pseudomonas* isolates were isolated from clay soil and primarily identified by morphological, physiological and biochemical characters (Table 1). For morphological characters**,** all isolates showed circular colony, rod cell shape, motile, negative reaction for gram stain, positive fluorescent pigment and variable colour from bright to light green for PS1, PS2, PS4 and PS5 and white-yellow for PS3, PS6 and PS7. Furthermore, bacterial isolates showed variable results for physiological and biochemical characters (Table 1).

Comparing these characters with those given in Bergey's Manual of Determinative Bacteriology

[39], the isolates were presumptively identified as *Pseudomonas* sp.

3.2 Greenhouse Experiment

3.2.1 Inoculation effects on common bean growth

Length, depth and dry matter within shoot and root of plants following co-inoculation with *R. leguminosarium sembiovar phaseoli* (TAL-3612) and different isolates of *Pseudomonas* measured at 45 days of growth was variable when common bean was grown in Leonard's jars (Fig. 1).

Generally, co-culture of *Rhizobium* and different isolates of *Pseudomonas* increased length of shoot, depth of root and dry biomass of their compared to control and single inoculation of *Rhizobium*. Inoculation of *Rhizobium* + PS7 isolate was significantly enhanced the length of shoot (22.68 cm plant^{-1}), root depth (10.98 plant^{-1}), dry biomass of shoot and root each by (5.66 and 2.76 g plant^{-1}) at 45 days compared to control plants. On the contrary, the single inoculation of *Rhizobium* accelerated length of shoot, depth of root and dry biomass of shoot and root by 19.46 cm plant^{-1}, 7.46 cm plant^{-1} and 4.95 g plant^{-1} and 1.90 g plant^{-1}, compared to un-inoculated plants (control), respectively.

The co-inoculation effect of *R. leguminosarum* and *Pseudomonas* on growth characteristics of common bean plants was variable. Observation of significant increase in the length of shoots, depth of roots and dry matter accumulation due to the amounts of ACC deaminase, synthesized by *Pseudomonas* and *R leguminosarum* [52], induce metabolic changes [53] and increase the growth of plants [21,54,55]. Besides these, ethylene also affects many physiological processes, such as leaf senescence, leaf abscission [56].

Table 1. Morphological, physiological and biochemical characters of bacterial isolates

No.	Isolate	Morphological characters					
		Colony	Colour	Cell shape	Motility	Gram reaction	fluorescent pigment
1	PS1	Circular	Bright green	Rod	Motile	-	+
2	PS2	Circular	Light green	Rod	Motile	-	+
3	PS3	Circular	White-yellow	Rod	Motile	-	+
4	PS4	Circular	Bright green	Rod	Motile	-	+
5	PS5	Circular	Bright green	Rod	Motile	-	+
6	PS6	Circular	White-yellow	Rod	Motile	-	+
7	PS7	Circular	White-yellow	Rod	Motile	-	+

No.	Isolate	Physiological characters						
		Glucose utilization	Ammonification	Nitrate reduction	Tolerance of NaCl at		Growth at	
					5 %	7 %	4°C	41°C
1	PS1	+	+	+	+	-	+	-
2	PS2	+	+	+	-	-	-	+
3	PS3	+	+	+	-	-	+	-
4	PS4	-	+	-	+	-	+	-
5	PS5	+	+	+	+	-	-	+
6	PS6	+	+	+	-	-	-	+
7	PS7	+	+	-	+	-	+	-

No.	Isolate	Biochemical characters					
		Oxidase	Catalase	Urease	Indole production	Casein hydrolysis	Starch hydrolysis
1	PS1	+	+	+	+	+	-
2	PS2	+	+	+	-	-	-
3	PS3	-	+	-	+	-	-
4	PS4	+	+	+	-	-	-
5	PS5	-	+	+	+	+	-
6	PS6	+	+	-	-	+	-
7	PS7	+	+	-	-	-	-

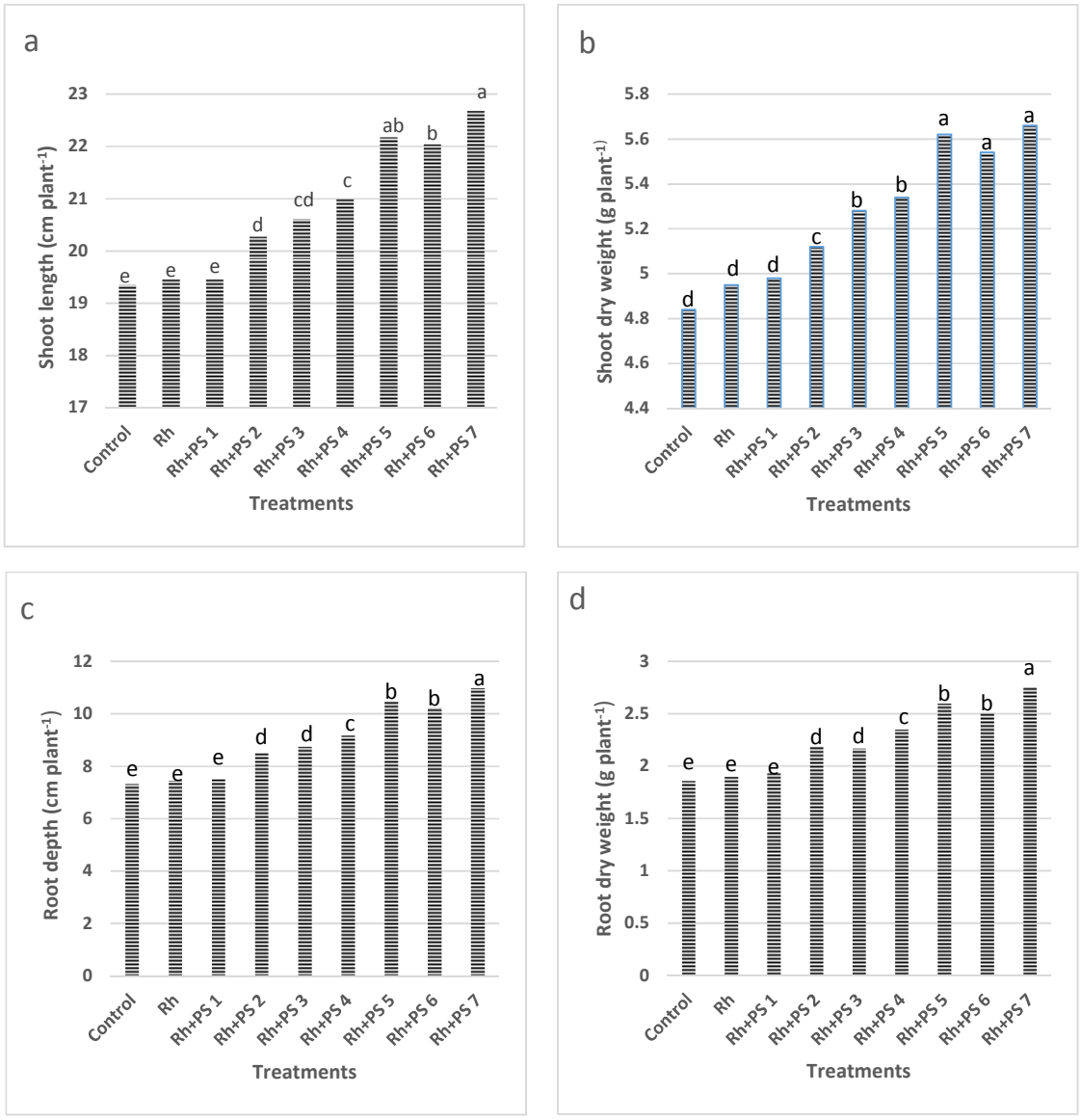

Fig. 1. Co-inoculation effects of *Rhizobium leguminosarium sembiovar phaseoli* (TAL-3612) and different isolates of *Pseudomonas* on a) length of shoot (cm plant^{-1}), b) shoot dry weight (g plant^{-1}), c) root depth (cm plant^{-1}), and d) root dry weight (g plant^{-1}) in common bean plants grown in greenhouse conditions. Mean values are significant at P ≤ 0.05

In agreement to this finding, plants inoculated with *R leguminosarum* and *Pseudomonas* have also shown dramatic increase in plant growth [12,24,31,33,35].

3.2.2 Inoculation effect on common bean symbiosis, photosynthetic and nitrogen content

Generally, the inoculation of *Rhizobium* used either alone or in combination with isolates of *Pseudomonas* were increased significantly (P ≤ 0.05).

The formation and distribution of nodules on *R. leguminosarium sembiovar phaseoli* (TAL-3612) and different isolates of *Pseudomonas* inoculated common bean plants was variable (Fig. 2). A maximum number of nodules (36.33 nodules plant^{-1}) and (32.66 nodules plant^{-1}) were formed on the root systems of common bean plants when inoculated with co- cultures of PS7

and PS6 isolates and *R. leguminosarum* grown for 45 days in pot experiment, respectively. Similar trend was also exhibited in nodules dry weight parameter.

Among co-culture of *R. leguminosarium sembiovar phaseoli* (TAL-3612) and different isolates of *Pseudomonas*. PS7 isolate had maximum positive effect and increased which recorded (45 and 248 mg plant^{-1}), followed by PS6 isolate (43 and 241 mg plant^{-1}) as compared to control (29.66 and 180 mg plant^{-1}) for total chlorophyll content and nitrogen content parameters, respectively. Also, plants co-inoculated with *Rhizobium* and *Pseudomonas* isolates accumulated significantly more shoot N content than those inoculated with *Rhizobium* alone.

Recently, most of research is focusing on increasing the legume–rhizobia symbiosis with inoculation of legumes with rhizosphere bacteria. Generally, co-inoculation of PGPR with *Rhizobium* species is a valuable, relevant, profitable, efficient, and environmentally friendly tool, which increased Biological Nitrogen Fixation (BNF), growth and productivity.

Previous studies have demonstrated that PGPR co-inoculation can enhance early nodule initiation and development on soybean [11], Pea [25], Common bean [5], and Lucerne [35].

Other reports have also shown positive results of inoculations of *Pseudomonas* spp. on nodulation, growth and productivity of legumes could be due to production of phytohormones such as auxin, gibberellins and cytokinins has been reported as a mechanism used to enhance nodule formation [12,57,58].

The increased of total chlorophyll content and nitrogen content in plant leaves as the result of bacterial isolates co-inoculation due to the increased plant nutrition and photosynthesis [10, 12,26].

3.3 Molecular Identification

The bacterial strain (PS7) was identified according to previous described morphological, physiological, biochemical characters and plant test in Leonard jars as well as using analysis of 16S rRNA. According to 16S rRNA analysis, the phylogenetic tree of the isolated bacteria (PS7) and related bacterial species based on the 16S rRNA sequence is shown in Fig. 3. It can be clearly seen that the isolated bacteria was included in the genus *Pseudomonas* and closely related to the species *Pseudomonas koreensis*. It showed the highest sequence similarities with *Pseudomonas koreensis* (95%). Sequence data were submitted to GenBank and it provided a GenBank accession number MG209738.

Of these, *Pseudomonas koreensis* MG209738 was selected with *R. leguminosarium sembiovar phaseoli* (TAL-3612) for studying the ability of co-inoculation to improve nodulation, growth and yield of common bean plants under lyzemeter conditions.

3.4 Lyzemeter Experiment

3.4.1 Inoculation effects on common bean growth and symbiosis

Four treatments (T_1: control, T_2: inoculation with *P. koreensis* MG209738, T_3: inoculation with *R. leguminosarium sembiovar phaseoli* (TAL-3612) and T_4: co-inoculation with *Rhizobium leguminosarium sembiovar phaseoli* (TAL-3612) and *Pseudomonas koreensis* MG209738) were studied in the present investigation to evaluate growth, nodulation and yield of common bean under lyzemeter experiment conditions at 30, 60 and harvest.

As shown in Table 2, the inoculation with the varied bio-inoculants gave significant differences than un-inoculated plants. As seen at 30 days, the treatments T_4, and T_3 recorded the highest plant height (30.33 and 29.00 cm plant^{-1}), number of nodules (37.00 and 28.66 nodule plant^{-1}), dry weight of plant (7.54 and 7.25 g plant^{-1}) and dry weight of nodules (0.313 and 0.226 g plant^{-1}), respectively as compared to control (T_1). Similarly at 60 days, co-inoculation treatments significantly enhanced growth parameters of plant. Thus, the increased in plant height, nodulation and dry biomass observed on co-inoculated in this study further supports the data demonstrating that root infection occurred earlier with co-inoculation.

The observation that were often present in pairs and appeared to have early signs of cluster-like morphology was mirrored by observations that there were often large cluster-like nodules on the co-inoculated plants. Also, co-inoculation exert beneficial effects on growth of plants by producing phytohormones (*i.e*. auxins, gibberellic acid, cytokinins and abscisic acid etc.), increasing solubility and availability of nutrients.

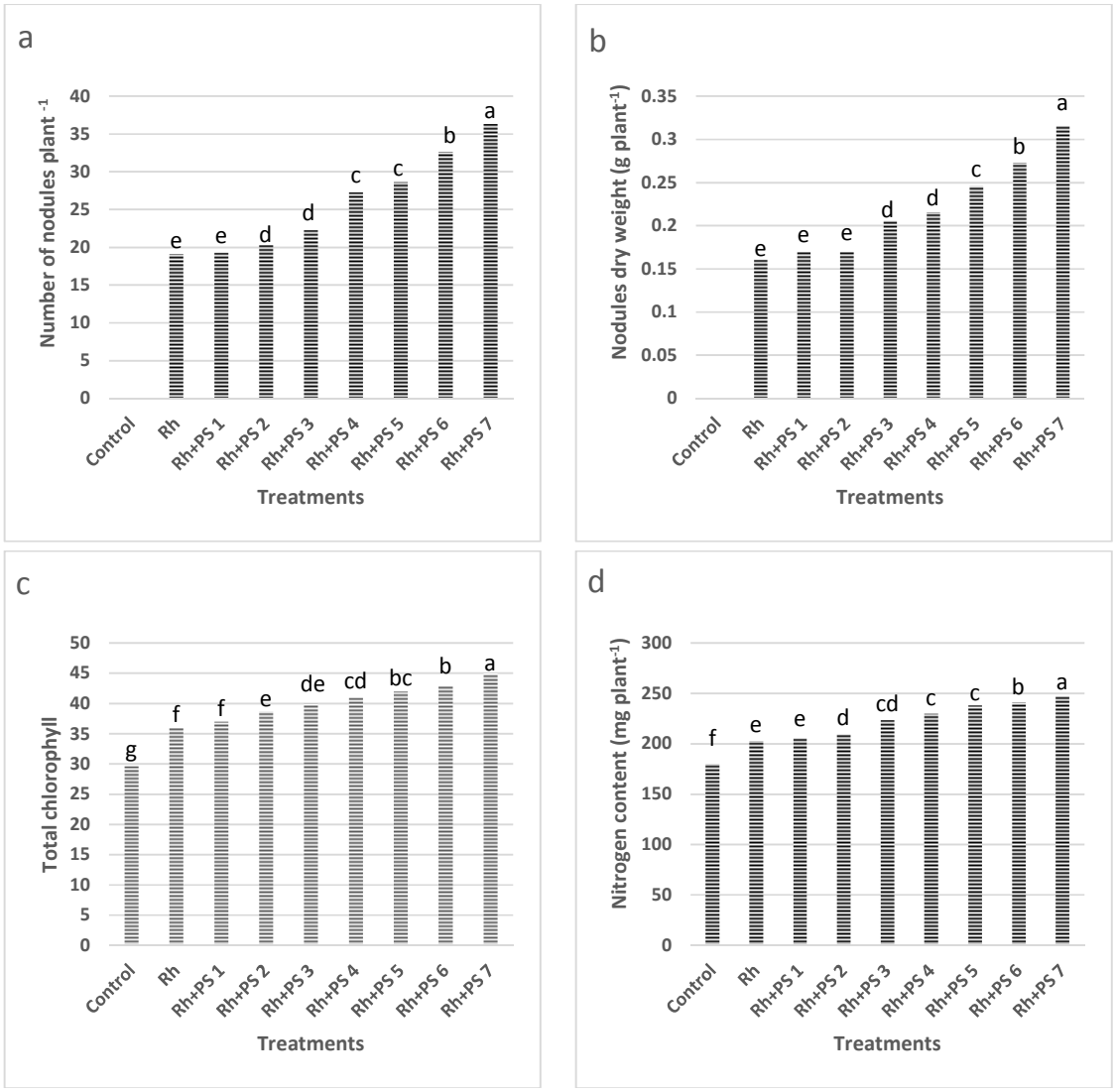

Fig. 2. Co-inoculation effects of *Rhizobium leguminosarium sembiovar phaseoli* (TAL-3612) and different isolates of *Pseudomonas* sp. on a) number of nodules, b) nodules dry weight (g plant^{-1}), c) total chlorophyll, and d) nitrogen content (mg plant^{-1}) in common bean plants grown in greenhouse conditions. Mean values are significant at P ≤ 0.05

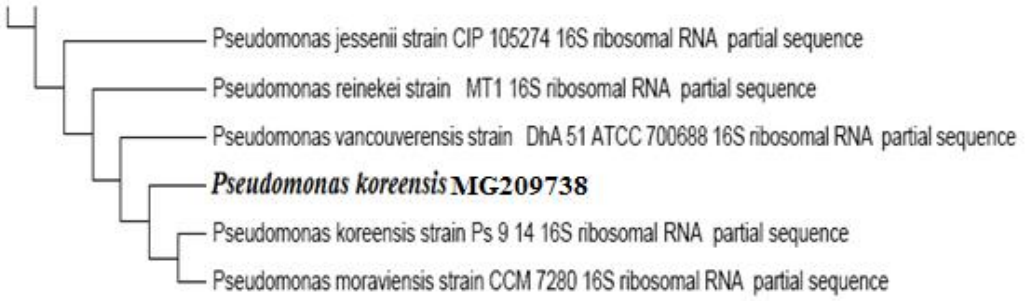

Fig. 3. Neighbor-joining phylogenetic tree reconstructed on the basis of 16S rRNA gene sequence (1.5 kb) showing the phylogenetic *Pseudomonas koreensis* MG209738

Table 2. Single and co-inoculation effect with *Rhizobium leguminosarium sembiovar phaseoli* (TAL-3612) and *Pseudomonas koreensis* MG209738 on plant height (cm), number of nodules and dry biomass of common bean plants at 30 and 60 days

Treatment	Plant height (cm)	Number of nodules plant^{-1}	Dry weight of plant (g plant^{-1})	Dry weight of nodules (g plant^{-1})
		30 days		
T_1	25.00 ^{c}	3.00 ^{d}	6.25 ^{c}	0.096 ^{d}
T_2	28.00 ^{b}	8.33 ^{c}	7.00 ^{b}	0.170 ^{c}
T_3	29.00 ^{ab}	28.66 ^{b}	7.25 ^{ab}	0.226 ^{b}
T_4	30.33 ^{a}	37.00 ^{a}	7.54 ^{a}	0.313 ^{a}
LSD (0.05)	1.718	2.771	0.437	0.031
		60 days		
T_1	29.66 ^{d}	6.66 ^{d}	7.42 ^{d}	0.12 ^{d}
T_2	32.00 ^{c}	12.33 ^{c}	7.98 ^{c}	0.19 ^{c}
T_3	36.00 ^{b}	34.66 ^{b}	8.98 ^{b}	0.30 ^{b}
T_4	40.00 ^{a}	46.33 ^{a}	10.03 ^{a}	0.39 ^{a}
LSD (0.05)	1.718	3.169	0.460	0.032

In a column means followed by a common letter are not significantly different at 5% level by DMRT, T_1: Control; T_2: inoculation with P. koreensis MG209738; T_3: inoculation with R. leguminosarium sembiovar phaseoli (TAL-3612); T_4: Co-inoculation with P. koreensis MG209738 and R. leguminosarium sembiovar phaseoli (TAL-3612).

These observations coupled with the data showed by [24,25,35,37,59,60,61].

3.4.2 Inoculation effect on nutrient uptake of common bean plants

The inoculation of *R. leguminosarum* and *P. koreensis* MG209738 and their mixture led to increases in macroelements content (N, P and K) and microelements content (Zn, Mn, Fe and Cu) over control (Table 3).

The best treatment gave high contents of N, P and K was T_4 treatment (co-inoculation with *P. koreensis* MG209738 and *R. leguminosarium sembiovar phaseoli* (TAL-3612)) which attained 262.33, 21.69 and 285.33 (mg plant^{-1}), while the treatment of control (chemical fertilizer) exhibited the lowest levels 194.66, 19.72 and 254.33 (mg plant^{-1}), respectively, with significant differences at 30 days of sowing. Similarly trend are showed at 60 days.

On the other hand, application of dual inoculation led to further increases of microelements contents of common bean plants as compared to single inoculation and control. T_4 treatment (co-inoculation) gave the highest values which recorded 37.17, 27.37, 51.79 and 4.17 (ppm plant^{-1}) at 30 days, and 51.95, 40.34, 94.68 and 7.73 (ppm plant^{-1}) at 60 days for Zn, Mn, Fe and Cu, respectively, as compared to single inoculation treatments (T_2 and T_3) and control.

It is important to mention here that the direct mechanisms of plant growth promotion by *Rhizobium* and *Pseudomonas* can be improved nitrogen fixation and solubilization of minerals from soil. Availability of microelements in soil by PGPR could be due to enhance in nodule initiation, number and size of nodules, nodule development, N_2-fixation rate, increasing photosynthetic pigments and play an important role involved in leghaemoglobin synthesis [37,60, 61].

Several studies showed the improvement of legume symbiosis and availability of microelements in soil by PGPR, due to enhance nodulation, nitrogen fixation and plant productivity in Common bean [10]; Pea [25]; Soybean [11]; Common bean [12]; Chickpea [33]; and Lucerne [35].

3.5 Enzyme Activity

Data of Table 4 revealed an increase in dehydrogenase and phosphatase activities with the application of the different treatments in rhizosphere of common bean plants at 30, 60 and harvest. The dehydrogenase activity was noted to decline with increasing plant age. In general, the treatments T_4 and T_3 recorded the highest values at all growth stages, they recorded 241.00 and 225.66 µg TPF g^{-1} soil day^{-1} at 30 days and 249.33 and 235.00 µg TPF g^{-1} soil day^{-1} at 60 days and 203.66 and 179.66 µgTPF g^{-1} soil day^{-1} at harvest.

At 30 days after sowing, phosphatase activity of soil recorded the highest values (6.50 µg g^{-1} soil

h^{-1}) as a result of Co-inoculation with *P. koreensis* MG209738 and *R. leguminosarium sembiovar phaseoli* (TAL-3612) significantly superior over all seed inoculation treatments and control (2.93 µg g^{-1} soil h^{-1}). While, at 60 days, phosphatase activity of plant rhizosphere showed that T_4 and T_3 treatments recorded 7.73 and 6.20 µg g^{-1} soil h^{-1} then decreased to 3.10 and 3.00 20 µg g^{-1} soil h^{-1} at harvest. Phosphatase activity in soil generally reduced with the aging of the plant.

In the entire crop period, the enzyme activity increased initially at 30 and 60 days and then declined with the age of the crop. These observations are in accordance with the findings of the present investigation.

Several studies showed that increases in enzyme activity has mainly due to the higher microbial population and the potential capacity of soil to perform biological transformations of importance to soil fertility [12,25,37].

Table 3. Single and co-inoculation effect with *Rhizobium leguminosarium sembiovar phaseoli* (TAL-3612) and *Pseudomonas koreensis* MG209738 on content of some nutrient uptake of common bean plants at 30 and 60 days

Treatment	Macro elements (mg plant^{-1})			Micro elements (ppm plant^{-1})			
	N	P	K	Zn	Mn	Fe	Cu
			30 days				
T_1	194.66 ^{d}	19.72 ^{c}	254.33 ^{a}	23.44 ^{d}	16.18 ^{d}	34.70 ^{d}	3.22 ^{d}
T_2	223.66 ^{c}	20.68 ^{b}	261.66 ^{a}	27.23 ^{c}	19.16 ^{c}	38.84 ^{c}	3.55 ^{c}
T_3	247.66 ^{b}	21.15 ^{ab}	271.00 ^{a}	33.41 ^{b}	23.94 ^{b}	46.90 ^{b}	3.82 ^{b}
T_4	262.33 ^{a}	21.69 ^{a}	285.33 ^{a}	37.17 ^{a}	27.37 ^{a}	51.79 ^{a}	4.17 ^{a}
LSD (0.05)	13.84	0.70	50.38	2.92	2.82	3.82	0.12
			60 days				
T_1	222.00 ^{d}	21.02 ^{d}	307.66 ^{d}	35.97 ^{d}	26.98 ^{d}	80.33 ^{b}	6.51 ^{d}
T_2	237.00 ^{c}	21.55 ^{c}	326.66 ^{c}	40.20 ^{c}	32.10 ^{c}	90.73 ^{a}	6.92 ^{c}
T_3	248.66 ^{b}	22.00 ^{b}	384.33 ^{b}	45.90 ^{b}	37.81 ^{b}	91.14 ^{a}	7.24 ^{b}
T_4	282.00 ^{a}	22.42 ^{a}	371.66 ^{a}	51.95 ^{a}	40.34 ^{a}	94.68 ^{a}	7.73 ^{a}
LSD (0.05)	8.43	0.21	12.17	4.01	2.38	4.07	0.25

In a column means followed by a common letter are not significantly different at 5% level by DMRT, T_1: Control; T_2: inoculation with P. koreensis MG209738; T_3: inoculation with R. leguminosarium sembiovar phaseoli (TAL-3612); T_4: Co-inoculation with P. koreensis MG209738 and R. leguminosarium sembiovar phaseoli (TAL-3612)

Table 4. Single and co-inoculation effect with *Rhizobium leguminosarium sembiovar phaseoli* (TAL-3612) and *Pseudomonas koreensis* MG209738 on dehydrogenase activity (µg TPF g^{-1} soil d^{-1}) and phosphatase activity (µg pnp g^{-1} soil h^{-1}) in rhizosphere of common bean plants at 30, 60 and harvest

Treatment	Dehydrogenase activity (µg TPF g^{-1} soil d^{-1})		
	30 days	60 days	At harvest
T_1	174.00 ^{d}	189.33 ^{d}	122.33 ^{d}
T_2	205.66 ^{c}	212.33 ^{c}	145.66 ^{c}
T_3	225.66 ^{b}	235.00 ^{b}	179.66 ^{b}
T_4	241.00 ^{a}	249.33 ^{a}	203.66 ^{a}
LSD (0.05)	9.58	6.85	12.50
Treatment	Phosphatase activity (µg pnp g^{-1} soil h^{-1})		
	30 days	60 days	At harvest
T_1	2.93 ^{d}	3.36 ^{d}	1.83 ^{c}
T_2	5.30 ^{c}	5.83 ^{c}	2.53 ^{b}
T_3	5.80 ^{b}	6.20 ^{b}	3.00 ^{a}
T_4	6.50 ^{a}	7.73 ^{a}	3.10 ^{a}
LSD (0.05)	0.40	0.33	0.20

In a column means followed by a common letter are not significantly different at 5% level by DMRT, T_1: Control; T_2: inoculation with P. koreensis MG209738; T_3: inoculation with R. leguminosarium sembiovar phaseoli (TAL-3612); T_4: Co-inoculation with P. koreensis MG209738 and R. leguminosarium sembiovar phaseoli (TAL-3612).

Table 5. Single and co-inoculation effect with *Rhizobium leguminosarium sembiovar phaseoli* (TAL-3612) and *Pseudomonas koreensis* MG209738 on number of pods, pod yield (g plant^{-1}), seed yield (ton ha^{-1}), total carbohydrate (%) and protein (%) of common bean plants

Treatment	Number of pods	Pod yield (g plant^{-1})	Seed yield (ton ha^{-1})	Total carbohydrate (%)	Protein (%)
T_1	15.00 ^{c}	224.66 ^{d}	3.68 ^{d}	48.33 ^{c}	18.00 ^{c}
T_2	17.66 ^{b}	247.00 ^{c}	4.90 ^{c}	51.66 ^{b}	20.66 ^{b}
T_3	20.66 ^{a}	268.66 ^{b}	5.33 ^{b}	53.00 ^{b}	22.66 ^{a}
T_4	22.00 ^{a}	298.66 ^{a}	5.53 ^{a}	55.33 ^{a}	23.66 ^{a}
LSD (0.05)	1.53	6.58	0.133	2.10	1.88

In a column means followed by a common letter are not significantly different at 5% level by DMRT, T_1: Control; T_2: inoculation with P. koreensis MG209738; T_3: inoculation with R. leguminosarium sembiovar phaseoli (TAL-3612); T_4: Co-inoculation with P. koreensis MG209738 and R. leguminosarium sembiovar phaseoli (TAL-3612).

3.6 Seed Yield and Quality Assessment

Data presented in Table 5 (above) indicated that co-inoculation with the different bio-inoculants variably increases number of pods, pod yield (g plant^{-1}), seed yield (ton ha^{-1}), total carbohydrate (%) and protein (%) of common bean plants.

Number of pods plant^{-1} was significantly increased due to combined inoculation with *P. koreensis* MG209738 and *R. leguminosarium sembiovar phaseoli* (TAL-3612). Statistically, significant results were recorded in T_4 treatment which showing number of pods (22.00 plant^{-1}) which was significantly superior over control (15.00 plant^{-1}). On the other hand, the maximum values of pod yield (g plant^{-1}) was observed in T_4 followed by T_3 and T_2 (298.66, 268.66 and 247.00 g plant^{-1}), respectively compared to chemical fertilizer (control). For seed yield (ton ha^{-1}), the treatments T_4 and T_3 attained the highest seed yield (5.530 and 5.330 ton ha^{-1}) compared to T1 (control), 3.680 ton ha^{-1}, respectively. The results of total carbohydrate and protein (%) in seeds of common bean showed significant variations under co-inoculation, which recorded the higher values in T_4 treatment 55.33 and 23.66% compared to 48.33 and 18.00% for T_1 (control), respectively.

In general, the maximum increase in yield parameters due to combined inoculation of PGPR was documented and it is important to estimate carbohydrate and protein contents in the yielded seeds of common bean plant for seed quality assessment.

These results was confirmed with those of other researchers who reported that increasing number of pods, pod yield (g plant^{-1}), seed yield (ton ha^{-1}), total carbohydrates and protein in the yielded seeds with using different PGPR [37,62,63,64].

4. CONCLUSION

This study confirms that the selected *Pseudomonas* can improve the growth, nodulation, nitrogen fixation and increase yield parameters of common bean plants inoculated with *Rhizobium leguminosarium sembiovar phaseoli* (TAL-3612).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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