Growth promotion and protection against root rot of sugar beet (*Beta vulgaris* L.) by two rock phosphate and potassium solubilizing *Streptomyces* spp. under greenhouse conditions

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Abstract

Purpose: Phosphorus (P) and potassium (K) shortages inhibit crop production, and soil borne plant diseases such as root rot by *Fusarium* spp. can cause extensive damage to crops. *Streptomyces bellus* (SB) and *S. saprophyticus* (SS) solubilize P and K and inhibit sugar beet (*Beta vulgaris* L.) associated Fusarium strains in laboratory conditions. *Methods*: To address their performance in vivo, their effects on sugar beet growth and root rot development was tested. *Results*: The tested strains showed a significant beneficial effect on growth and yield parameters of sugar beet when mixed in the soil with insoluble natural rock phosphate (RP) and/or K mineral orthoclase (OT). Compared to the non-inoculated treatment, the highest shoot and root dry biomass were recorded with RP+OT+SB. Highest P and K levels in leaves were with OT+SB and RP+SB, and the treatment RP+OT+SB increased both soil P and K. Interestingly, these SS and SB strains exhibited high protection effects of 100% and 75%, respectively, when the soil was infested by *F. equiseti* and *F. fujikuroi*, causal agents of root rot disease of sugar beet in Beni Mellal region. *Conclusion*: These results can be exploited to mitigate the detrimental impacts of nutrient limitation for and disease susceptibility of sugar beet.

Keywords: Rock phosphate, orthoclase, biofertilisers, *Streptomyces*, sugar beet growth, biocontrol, root rot, greenhouse conditions.

Introduction

Phosphorus (P) and potassium (K) are major nutrients that play key roles in plant growth and development (Alori et al. 2017; Parmar and Sindhu 2018), and the crucial role of P and K in improving the yield and quality of sugar beet is well established (Zengin et al. 2009; Mekdad et al. 2021). Therefore, P application enhances root system development, plant growth and carbon allocation to roots in sugar beet (Madani et al. 2017; Ghaly et al. 2019). P is also involved in the synthesis of lipids and nucleic acids (Makhlouf et al. 2020). By contrast, K contributes in several essential functions such as biosynthesis and transfer of sucrose to storage roots, osmotic potential regulation and regulation of water uptake in sugar beet (Barlog et al. 2013; Hanafy et al. 2019). Furthermore, Fathy and Attia (2016) reports that the sucrose percentage, yield quality and juice purity of sugar beet are correlated positively with K uptake.

Excess use of chemical fertilizers can lead to negative environmental impacts such as groundwater pollution, eutrophication of rivers and affect human health (Naik et al. 2019). In acidic soils, reactive rock phosphate (RP) can be partially solubilized making then P available for plant use. Therefore, direct application of reactive RP can constitute an ecofriendly alternative to P chemical fertilizers. Unfortunately, there is a lack of technologies that make RP applicable in alkaline soils (Maharana et al. 2021). Potassium exists in soil in various mineral forms such as orthoclase, silicate, biotite, muscovite, feldspar, mica and vermiculite (Sattar et al. 2018). However, 90 to 98% of this K is not available for plant use (Etesami et al. 2017).

Considering the environmental risk associated with excess fertilization practices, a current research priority is to achieve low-input agricultural systems with reduced inputs of fertilizers and pesticides, strongly relying on soil biota (Jacoby et al. 2017).

P and K solubilizing soil microorganisms have been recognized to improve plant nutrient uptake (Sattar et al. 2018; Soumare et al. 2020). Several rhizosphere bacteria use RP and mineral potassium as substrates (Han and Lee 2006; Bagyalakshmi et al. 2017; Meena et al. 2018; Maharana et al. 2021). In particular Actinobacteria are of special interest with their ability to solubilize P and K from rock minerals (Hamdali et al. 2008a, 2012; Liu et al. 2016; Han et al. 2018; Boubekri et al. 2021; Hamdali et al. 2021). Furthermore, this group of filamentous bacteria produces a large range of antibiotics against phytopathogenic fungi (Hamdali et al. 2008b, c, 2021; Hamid et al. 2020; Zhang et al. 2020). Several studies have reported the efficiency of Actinobacteria against root rot caused by *Fusarium* sp. in different plants such as cucumber (Hatamy et al. 2014), Wheat (Orakçı et al. 2010), tomato (Goudjal et al. 2016) and sugar beet (Diep and Hieu 2013; Aallam et al. 2021b). In contrast, only few studies investigating the P and K Solubilizing Actinobacteria (PKSA) have been reported (Han et al. 2018; Nafis et al. 2019; Aallam et al. 2021a, b; Boubekri et al. 2021), at the in vitro level. To the best of our knowledge, there are no reports describing PKSA with their both P and K biofertilization and biocontrol of root rot disease of sugar beet in pot experiments.

In this regard, the effects of two preselected PKSA *Streptomyces bellus* (AYD) and *Streptomyces saprophyticus* (DE2) (Aallam et al. 2021a, b) on sugar beet growth and tolerance to pathogen infection were evaluated. For this purpose, sugar beet seedlings were cultivated in greenhouse using soils supplemented with RP and/or orthoclase, and grown in the presence and absence of root rot causing *Fusarium* spp. Previous work considering the performance of these two bacteria *in vitro* suggested (Aallam et al. 2021a, b) that of the two isolates, *S. bellus* has higher in vitro mineral P and K solubilization activities auxin production level and antifungal activity against *Fusarium* spp. than *S. saprophyticus*.

This work was based on the following hypotheses: i) *Streptomyces bellus* promotes sugar beet growth and yield more strongly than *S. saprophyticus*. Since both strains solubilize both P and K minerals, we expected that ii) the positive impact of the bacteria on plant growth and P and K concentration is at its strongest in the combined treatment with rock phosphate and orthoclase. And due to their inhibitory activity against *Fusarium* isolates *in vitro*, we hypothesized that iii) both *Streptomyces* strains suppress root infection by *Fusarium* in sugar beet plants.

Materials and methods

Soil sampling and analysis

Soil samples were previously collected from a non-fertilized sugar beet field located 40 km south of Beni Mellal region of Morocco in June 2017 (Aallam et al. 2021a). According to the FAO-UNESCO (1990) classification, the soil is a calcareous calcisol with low available P and K contents. The soil samples were collected from 0 to 10 cm depth after removing 3 cm of the soil surface, air dried, homogenized, sieved (<2 mm), mixed with sand at 3:1 (v/v) (Chandra et al. 2019) and sterilized at two successive days for 2 h at 121 °C in order to eliminate autochthon microorganisms. The soil was used for further experiments within 48 hours.

The physicochemical characteristics of the soil were previously described as follows: pH (H_2O) 7.49; pH (KCl) 7.09; Organic matter 4%; Mineral matter 88%; Water content 7.2%; Electrical conductivity 0.25 µS/cm; total nitrogen 2.33%; soluble phosphate PO_4^{3-} 0.34%; Mg^{2+} 2.22%; exchangeable potassium K^+ 0.77%; and MnO 0.04% (Aallam et al. 2021a). The molted rock phosphate used as a supplement is a calcium hydroxyapatite constituted by O: 56.53%; F: 2.42%; Na: 1.81%; Mg: 1.94%; Al: 2.03%; P: 9.37%; S: 0.77%; Sn: 0.12%; Ca: 16.35%; Fe: 0.60% (Hamdali et al. 2008a). The orthoclase powder used as a supplement constituted by K^+: <0.1%; PO_4: 0.07%; SiO_2: 78.14%; Na_2O: 0.10%; Al_2O_2: 13.76%; Fe_2O_3: 0.21%; Ca^{++}: 0.41%; Mg^{++}: <0.1%; MnO: <0.01%; TiO_2: 0.4%; S: 0.04% (Hajjaji et al. 2008).

Actinomycetes and preparation of the inocula

Streptomyces bellus MW797036 (SB) and *Streptomyces saprophyticus* MW797316 (SS) isolated from sugar beet fields were previously selected for their multiple Potential Plant Growth (PGP) abilities (Aallam et al. 2021a, b); the terms MW797036 and MW797316 represent Genbank accession numbers of the partial SB and SS 16S rRNA gene sequences. Of note, the name *Streptomyces saprophyticus* has not been validly published under the rules of the International Code of Nomenclature of Bacteria (Bacteriological Code). Spores of these strains, stored in 20% sterile glycerol at 20 °C, were used to inoculate (at 10^6 spores mL^{-1}) 50 mL cultures of liquid Bennett medium (Jones 1949) incubated in 250 mL Erlenmeyer flasks for 3 days at 28 °C under constant agitation on a rotary shaker (New Brunswick Innova 2000, New Jersey, USA) (180 g min^{-1}). The *Streptomyces* mycelium was centrifuged at 10,000 × g for 10 min, washed twice with phosphate buffer saline (PBS; pH 7.2, 10 mM K_2HPO_4–KH_2PO_4, 0.14 M NaCl), fragmented through the needle of a sterile syringe and re-suspended in 10 mL of sterile deionised water. Five mL of the mycelial suspension was added to 2.5 g of wet carboxymethylcellulose (CMC, Merck, Darmstadt, Germany). This paste was then mixed with 50 g of surface sterilized sugar beet seeds. Each seed was coated by a thin layer of wet CMC, containing 10^6 colony forming units (cfu) bacteria, as determined by plating on Bennett agar.

Seed treatment and pot experiments

The experiments were conducted with sugar beet (*Beta vulgaris* L.), Macumba cultivar. The seeds were harvested in October 2019, and obtained from COSUMAR, the major Moroccan sugar manufacturer (www.cosumar.co.ma). Surface sterilization of the sugar beet seeds was achieved by soaking the seeds in a solution of 0.4% sodium hypochlorite and 0.1% Tween 80 for 5 min. Subsequently, the seeds were rinsed extensively with sterile deionized water and inoculated as described before.

Before sowing, surface sterilized plastic pots (14 cm diameter, 12 cm height) were filled with 1 Kg of the autoclaved soil. Seeds with or without inoculation (10^6 cfu per seed) were sown, five per pot, at 2 cm depth and watered every two days with 50 mL of sterile deionized water. After germination, the seeds were thinned to one plant per pot. The experiment was completely randomized and consisted of 12 treatments, as described in Table 1, with five replicates each. In all experiments, the sugar beet plants were grown in a greenhouse at 24°C under artificial light (≈ 140 µmol m^{-2} s^{-1}) for 16h and 17°C for 8h in darkness for two months.

Plant and soil analysis

The sugar beet plants were uprooted and the roots were washed to remove soil particles and organic debris. Shoots and roots were split off, fresh and dry (overnight at 105 °C) weights were measured and number of leaves was recorded. Shoot and root dry matter and length were determined for each plant for each treatment. Samples (plant and soil) were air dried and analyzed for total nitrogen using Kjeldahl method, total P and total K, available P content (Olsen-P) according to NF ISO 11263 and available K according NFX 31-103. N, P and K estimation was done using ICP-OES (Agilent 5110 ICP-OES, Santa Clara, USA).

Chlorophyll a, chlorophyll b and carotenoid contents were quantified according to the Arnon (1949). Sugar beet fresh leaves (between 0.25 and 0.5g) were left for 24 h at 4 °C in the presence of 80% acetone. After centrifugation (4000 g 10 min^{-1}), the absorbance was recorded at 470 nm, 537 nm, 647 nm and 663 nm using a UV-visible spectrophotometer (Macy(China) Instruments Inc, Shanghai, China). Chlorophyll a, chlorophyll b and carotenoids estimation (µmol g^{-1} FW) was done as described by Sims and Gamon (2002):

Chlorophyll a (µmol/g FW) = $((0.01373*A_{663})-(0.000897*A_{537})-(0.003046*A_{647}))$ Df * W

Chlorophyll b (µmol/g FW) = $(0.02405*A_{647} - 0.004305*A_{537} - 0.005507*A_{663})$ Df * W

Carotenoids (µmol/g FW) = ((A_{470} – (17.1 * (Chl a +Chl b) – 9.479 - anthocyanin) Df * W

Where:

Anthocyanin: 0.08173 * A_{537} – 0.00697 * A_{647} – 0.002228 * A_{663};

A_x: is the absorbance at wavelength x (nm); Df: means dilution factor (mL); W: weight of the leaf (g).

Fusarium spp. preparation and soil inoculation, and the greenhouse experiment

Efficiency of the two *Streptomyces* isolates against *F. fujikuroi* and *F. equiseti*, causal agents of rot root disease previously isolated from a contaminated sugar beet field in the Beni Mellal region (Aallam et al. 2021b), was evaluated in a greenhouse. Lawns of *Fusarium* strains were grown on the surface of Potato Dextrose Agar (PDA) medium (Merck, France) for 72 h. Then two agar plugs (10 mm diameter) were cut out and used to inoculate 50 mL cultures of PDA liquid medium in 250 mL Erlenmeyer flasks and grown at 28± 2 °C on a rotary shaker (180 g min^{-1}) for 48 h. The cultures were centrifuged at 10,000 g for 10 min, washed twice with PBS 10 mM and fragmented through the needle of a sterile syringe. In order to ensure infection, the mycelium of each *Fusarium* spp. was re-suspended in 50 mL of sterile PBS (0.25 mM) to give a less concentrated suspension (100 cfu mL^{-1}) as verified by plating the appropriate dilutions on PDA agar. The substrate for sugar beet cultures was the *Fusarium* contaminated sugar beet field soil of the Beni Mellal region. Surface sterilized plastic pots (7 cm diameter) were filled with 300 g autoclaved soil and infested or not with 1 mL of the appropriate dilution of each the two *Fusarium* spp. This corresponded to 3 cfu g^{-1} substrate.To start the greenhouse experiment, five *Streptomyces* inoculated or non-inoculated sugar beet seeds (10^6 cfu per seed) were sown in each pot at 2 cm depth. Three experimental situations were examined: (1) non-infested soil with non-inoculated seeds (control), (2) *Fusarium* spp. (*F. fujikuroi* or *F. equiseti*) infested soil with non-inoculated seeds, (3) *Fusarium* spp. (*F. fujikuroi* or *F. equiseti*) infested soil with inoculated seeds. There were five replicate pots per treatment. The pots were incubated for five weeks, with a 16 h photoperiod at 24 °C/17 °C (light at ≈ 140 µmol m^{-2} s^{-1} /dark). They were watered every two days with 50 mL of sterile deionized water. The sugar beet plants were uprooted; the roots were washed to remove soil particles and organic debris. Shoot and root lengths were determined for each plant. The plant infection symptoms by *Fusarium* spp., root staining, was assessed according to five categories: (0) no staining; presence of root staining in (1) 1-24%; (2) 25-49%; (3) 50-74% and (4) 75-100% of the roots.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) using SPSS software 20.0 package for Windows and significant differences between means were compared using Duncan's protected LSD test at $P < 0.05$. The percentages of healthy plants were arcsin ($\sqrt{x}$) transformed before statistical analysis. Superanova1 (Abacus Concepts Inc., Berkeley, CA, USA) was used for all analyses. Effect of the nutrient sources and inoculation with two tested strains on the growth of sugar beet was evaluated using PERMANOVA of the package *vegan* within R (R Core Team 2020). PERMANOVA analyses were conducted separately for sugar beet growth parameters including leaf number, shoot and root lengths and dry weights, and sugar beet physiological parameters including leaf chlorophyll a and b as well as carotenoid levels, and leaf P and K levels.

Results

Effect of the nutrient sources and inoculation with *Streptomyces bellus* (SB) or *S. saprophyticus* (SS) on the growth of sugar beet

Sugar beet was grown for 60 days in greenhouse with no nutrient addition, orthoclase (OT), rock phosphate (RP) and both nutrient sources (RP+OT), and the absence or presence of SB and SS (Fig. S1). PERMANOVA indicated significant nutrient source and bacterial inoculation dependent differences between growth and physiological properties of sugar beet. Significant effect by nutrients was observed for both plant growth ($P = 0.001$, $R2 = 0.30$: Fig. 1A) and plant physiological parameters ($P = 0.012$, $R2 = 0.20$; Fig. 1B), as well as by bacteria with ($P = 0.001$, $R2 = 0.22$; Fig. 1C) and ($P = 0.001$, $R2 = 0.22$; Fig. 1D).

In the absence of *Streptomyces* inoculation, plant growth parameters were at their lowest on non-supplemented original soil ($N_0P_0K_0$) which was regarded as a control treatment (Fig. 2). Seedling length increased with rock phosphate + orthoclase (RP+OT) and OT with significant differences compared to the control ($p > 0.05$) (Fig. 2D). Shoot biomass (Fig. 2A) increased from $N_0P_0K_0$ and RP by the isolate SB. Leaf number increased with OT, and RP+OT (Fig. 2C). Root biomass increased from RP by SS and from RPOT by SB (Fig. 2B).

Effect of *Streptomyces* on chlorophyll content

Generally, the application of actinomycetal inoculant show a significant effect on pigment content in sugar beet plants compared to the non-inoculated plants ($N_0P_0K_0$; RP; OT; RP+OT) (Fig. 3). The lowest concentration of chlorophyll a was

found in both treatments $N_0P_0K_0$ and RP+OT (0.55 µmol g^{-1} FW). Chlorophyll a contents in treatments where SS and SB combined with RP and/or OT were almost similar and ranged between 0.66 and 0.77 µmol g^{-1} FW (Fig. 3A). A clear variation of chlorophyll b content was observed after 60 days of sugar beet growth (Fig. 3B). The lowest values were recorded in presence of RP, OT, OT+SS and OT+SB and they ranged from 0.16 to 0.183 µmol g^{-1} FW. In contrast, both RP+SB and RP+SS treatments showed a high value of Chl b being 0.59 and 0.61 µmol g^{-1} FW, respectively. Generally, inoculation of both strains increased the amount of carotenoids compared to the non-fertilized and non-inoculated ($N_0P_0K_0$) plants (Fig. 3C). In presence of SB, carotenoid content ranged from 0.26 µmol/g FW in RP+SB and OT+SB and 0.29 RP+OT+SB. However, in presence of SS, the amount of carotenoids was ranged from 0.24 µmol g^{-1} FW in OT+SS and 0.3 µmol g^{-1} FW in RP+SS (Fig. 3C).

Effect of inoculation of strains on P and K contents in plant

As compared to the non-fertilized and non-inoculated ($N_0P_0K_0$) plants (2.5 mg g^{-1}), P content increased gradually in the treatments RP+OT, RP+SB, RP+SS and OT+SB which were 3.3, 3.4, 3.5 and 4.5 mg g^{-1}, respectively (Fig. 3D). K concentration in $N_0P_0K_0$ was 26.3 mg g^{-1}, and variable between the treatments (Fig. 3E). While inoculation with SS caused a shoot K content that ranged from 20.4 to 28.3 mg g^{-1}, higher values were detected especially in treatments inoculated with SB (RP+OT+SB, OT+SB, and RP+SB[MTt1]).

Effect of *Streptomyces* on N, P and K contents in soil

Sugar beet treatments with SB and SS led to higher soluble soil P and K contents. Combination of *S. bellus, S. saprophyticus,* RP and OT remarkably increased the total concentration of both P (RP+OT+SS: 2.1 g kg^{-1}, RP+OT+SB: 2.3 g kg^{-1}) and K (RP+OT+SS: 7.51 g kg^{-1}, RP+OT+SB: 7.60 g kg^{-1}) in the soil compared to the control (P: 1.15 g kg^{-1}, K: 7.1 g kg^{-1}). In contrast, RP+SS and OT+SB increased the available soil P content which was 0.05 g kg^{-1} and 0.06 g kg^{-1}, respectively as compared with $N_0P_0K_0$ (P: 0.04 g kg^{-1}). Moreover, available K in soil was increased due to the combination of SB with RP and SS with OT ($N_0P_0K_0$: 0.29 g kg^{-1}, RP+SB: 0.40 g kg^{-1}, OT+SS: 0.52 g kg^{-1}) (Table 2).

Suppression of *Fusarium* infection by the two *Streptomyces* spp.

When the mycelium of any of the two *Streptomyces* strains was used to coat the sugar beet seeds, the infection level of sugar beet roots and the *Fusarium*-mediated inhibition of sugar beet growth were reduced after five weeks of cultivation

(Fig. S2; Fig. 4). Treatment with the two strains *S. bellus* and *S saprophyticus* reduced the percentage of *Fusarium* infection and enhanced sugar beet growth (Fig. 4A, B). *Fusarium fujikuroi* (CH1 and CH3) and *F. equiseti* CH2 showed high percentages of root infection (highest mean value was for CH2 with 93.5%), compared to plants inoculated with CH1, CH2 or CH3 and with SB (mean 12%) or with SS (mean 6%). Sugar beet shoot (Fig. 4B) and root (Fig. 4C) length increased by SB and SS inoculation compared to *Fusarium* infection infested plants in the absence of the bacteria. Chlorophyll b and carotenoid contents stayed comparable between the treatments.

Discussion

In vivo results demonstrate that both tested *Streptomyces* isolates, SB and SS, promote the growth and improve the physiological parameters of sugar beet, and increase its tolerance against *Fusarium* root rot. They support the view that plant associated streptomycetes provide multiple plant beneficial traits (Hamdali et al. 2008b; Kurth et al. 2015; Yuan et al. 2015; Raymond et al. 2020; Worsley et al. 2020; Gebauer et al. 2021). As SB was the more efficient one of the two tested strains, its potential use for field applications especially warrants further attention.

According to the second hypothesis, SS and SB with RP and OT enhanced root dry matter, leaf number and shoot and root length of sugar beet compared to the non-inoculated treatments. This suggests that P and K mobilization may be the main means of plant growth promotion by these streptomycete strains. Similarly, inoculation of wheat seeds with *Micromonospora aurantiaca* and inoculation of walnut seeds with *Pseudomonas chlororaphis* and *Arthrobacter pascens* in the presence of RP promoted root and shoot growth (Hamdali et al. 2008b; Yu et al. 2012). Several studies have reported that bacteria support plant growth when rock P and rock K are applied to the substrate. These studies include wheat inoculated with *Bacillus* sp. MWT-14 (Tahir et al. 2018) and maize inoculated with *Agrobacterium tumefaciens* (Meena et al. 2018).

When RP and/or OT are supplemented to the substrate, the P and K contents of sugar beet plant are increased by SB or SS, in agreement with the results of Sacristán-Pérez-Minayo et al. (2020) using *P. fluorescens* and *P. chlororaphis*, as well as the results by Kaur and Reddy (2015) with *Pantoea cypripedii* and *Pseudomonas plecoglossicida*. Moreover, the content of available P in soil increased in the presence of SB with OT and in the presence of SS with RP, and the available K in soil in the presence of SS with OT or RP. This indicates that the two bacteria show complementary effects on mineral nutrient solubility, and the effects of combined SB and SS inoculation should be tested. Availabilities of P and K are considered very important to sugar beet growth, and these nutrients have alternative roles in plant physiology. P represents

often a limiting factor for sugar beet growth due to its central roles in e.g. energy transfer or as a constituent of nucleic acids and membranes (Hadir et al. 2021). Elhaissoufi et al. (2020) showed that inoculation of *Pseudomonas* spp. with RP increased not only P but also N content in shoots and roots of wheat plants, indicating that by P mobilization the assimilation of N by the plant can also be stimulated. It has been shown that inoculation of sugar beet with N_2-fixing and phosphate solubilizing *Bacillus* spp. increase root, leaf, and sugar yield (Sahin et al. 2004). Single *Bacillus* strain inoculations with N_2-fixing bacteria increased sugar beet root yield, and dual inoculation with a P solubilizing bacterium gave higher increases. This suggests that it should be tested in the field, if the two PSB of the present work, SB and SS, interact positively with N_2-fixing bacteria from sugar beet rhizosphere.

Neither SB nor SS showed a consistent positive effect on plant growth, P or K uptake. Instead, each bacterium showed P or K substrate specific effects on sugar beet, and consistent positive effects of both bacteria were merely found in regard to leaf chlorophyll a and carotenoid levels. We suggest that these inconsistencies most probably rely on feedback processes between shoot, roots, root exudates, soil, K or P sources, and *Streptomyces* isolates (Vetterlein et al. 2020). For instance, potassium application enhances soybean root exudation of phenolic acids and leads to the suppression of a cyst nematode (Gao et al. (2018), and increased soil phosphorus availability alters faba bean root exudation leading to stimulation of root growth and phosphorus uptake in a neighboring maize plant (Zhang et al. 2016). In general, changes in exudation are one major factor in the regulation of mycorrhiza formation or establishment of rhizobacteria, but in comparison to other rhizosphere bacteria, the streptomycetes are more likely to feed on complex organic matter than on root exudates (Worsley et al. 2021). Nevertheless, their physiology and nutrient acquisition potential are highly affected by P and K levels (Aharonowitz and Demain 1977; Chater et al. 2010), and our data suggest that P and K related changes strongly affect sugar beet-*Streptomyces* interactions.

On the other hand, K plays a role in photosynthesis and respiration as well as osmoregulation, including the reduction of the negative effects of drought stress (Aksu and Altay 2020; Hadir et al. 2021). It is established, that P and K solubilizing microorganisms can stimulate crop nutrition (Saleemi et al. 2017; Kour et al. 2020; Vasseur-coronado et al. 2021). Of note, the siderophores production potentials of SB and SS (Aallam et al. 2021a) and their positive influence on sugar beet chlorophyll levels, indicative of improved N nutrition, further support their role in improved sugar beet nutrition under nutrient deficiency.

Boubekri et al. (2021) have isolated *Streptomyces* spp. producing IAA and siderophores with a dual capacity of solubilizing mineral source of P and K (RP and mica powder) and improve the germination of wheat plants under greenhouse conditions. It was therefore plausible that the ability of the applied SB and SS to produce IAA (Aallam et al.

2021a, b) support sugar beet root proliferation. In support to this, an increase in root biomass was detected in RP by SS and in RP+OT by SB. *Variovorax paradoxus* NG-T6, *Micrococcus yunnanensis* TGT-R7 and *Planococcus rifietoensis* LH-T4 improve germination of sugar beet under salt stress (Zhou et al. 2017), and another interesting trait in light of field applications is improved stress tolerance. Namely the site of isolation in Morocco, Beni Mellal region, is characterized by high temperatures, intensive radiation and low soil moisture. Increase in carotenoid contents by SB and SS, antioxidative pigments which fulfill essential functions in photosynthesis for photosystem assembly, light harvesting, and photoprotection (Yuan et al. 2015), and the representation of ACC deaminase activity by rhizosphere streptomycetes (Gebauer et al. 2021) could also contribute to sugar beet growth under Beni Mellal region's field conditions.

There was a clear relationship between the recently reported (Aallam et al. 2021b) *in vitro* inhibitory activity against the *Fusarium* strains and the level of protection against root rot by SB and SS. This suggests a direct antagonism against Fusarium (Smith et al. 1990) plays a role in the inhibition of root rot by the bacteria. Nevertheless, the potential role of *Streptomyces* induced resistance (Lehr et al. 2008; Kurth et al. 2014), that was not assessed in this study, should not be neglected. The determinants of inhibition can be multiple. They comprise *Streptomyces* generated fungal cell wall degrading enzymes, chitinases or beta glucanases, and hydrogen cyanide (Sahin et al. 2004), and *Streptomyces* secondary metabolites such as the polyene antibiotic faeriefungin (Smith et al. 1990). Interestingly, Xiao et al. (2002) observed that the size of *in vitro* inhibition zones for individual *Streptomyces* isolates against specific pathogens was not significantly correlated with the extent of biocontrol of those pathogens. Instead, biocontrol activity correlated with seedling weights when inoculated with the *Streptomyces* strains in the absence and presence of the pathogens. Searching for effective *Streptomyces* spp. strains by in vitro inhibition can lead to effective protection under field conditions, with a decreased disease severity (Smith et al. 1990; Sahin et al. 2004; Colombo et al. 2019). Of note, the disease symptoms of *Fusarium* spp. in sugar beet are variable, including wilting of the foliage or interveinal chlorosis and necrosis (Burlakoti et al. 2012), interveinal yellowing or stunting (Hanson 2006), and root rot (Abada 1994). A longer pot experiment or field experiment should be conducted to evaluate the disease symptoms elicited by each Fusarium isolate, and to assess, SB and SS protect sugar beet plants from *Fusarium* disease symptoms.

Conclusions

The results confirmed at a greenhouse experimental level the in vitro observed capacities of P and K solubilization by SS, and SB to improve plant P nutrition when growing plants in soil according to the first hypothesis, in particular by SB. The performances of these bacteria against Fusarium infection further support both their potential for sugar beet growth

promotion, that were strongest in the combined treatment with rock phosphate and orthoclase. Future work will test if *Streptomyces bellus* and *Streptomyces saprophyticus* strains withhold their effectiveness under field conditions.

Declarations

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement

The raw data and additional figures are available in **Supplementary Material.**

Author contributions

HH, YA, MT and DD: conception and design of the study. HH and YA performed the experiments. YA, MT and TER performed the statistical analysis. YA, MT, TER and HH wrote the first draft of the manuscript. MT, SL, DD and AH wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

Supplementary material

The Supplementary Material for this article can be found online at:

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Captions

Figure 1. Overview on the effects of nutrient sources and *Streptomyces* bacteria on sugar beet growth and physiology. Principal components analysis of the combined variables of plant growth (leaf number, shoot and root dry weight, shoot and root length) and plant physiological variables (chlorophyll a, chlorophyll b, carotenoids, plant leaf P and K) of sugar beet plants without bacterial inoculation or with *S. bellus* (SB) or *S. saprophyticus* (SS), and in the presence of rock phosphate (RP) and/or orthoclase (OT) as the sole P and K source. Data points for each group represent individual pots. Based on a permutational multivariate analysis of variance of the combined variables of plant growth or plant physiological variables, R-squared (R^2) represents the proportion of the variance that is explained by the nutrient source or the bacterial inoculation, and P-value the corresponding significance level.

Figure 2. Growth parameters of sugar beet plants. (A) Shoot dry weight, (B) root dry weight, (C) leaf number, (D) shoot length and (E) root length of sugar beet plants without bacterial inoculation or with *S. bellus* (SB) or *S. saprophyticus* (SS), and in the presence of rock phosphate (RP) and/or orthoclase (OT) as the sole P and K source. $N_0P_0K_0$ marks the negative control. Boxplots represent three replicates after 60 days of cultivation. Different lowercase letters above bars shows significant differences between treatments within $p \leq 0.05$.

Figure 3. Plant physiological parameters of sugar beet plants. Mean values of (A): chlorophyll a, (B): chlorophyll b, (C): relative carotenoid content, (D): leaf P and (E): leaf K in sugar beet plants after inoculation of *S. bellus* (SB) and *S. saprophyticus* (SS) in presence of rock phosphate (RP) and/or orthoclase (OT) as sole source of P and K. N0P0K0: negative control; NPK: positive control. Values represent means of five replicates after 60 days of cultivation and errors bars represent standard deviation. Different lowercase letters above bars shows significant differences between treatments within $p \leq 0.05$.

Figure 4. Streptomycetes diminish sugar beet root infection and sugar beet growth inhibition by *Fusarium*. (A) Infection levels of sugar beet roots, (B) shoot and root lengths, (C) leaf numbers, and (D) Chlorophyll and carotenoid contents of sugar beet plants after five weeks of cultivation. Treatments include inoculations with the fungi CH1, *Fusarium fujikuroi*; CH2, *F. equiseti* and CH3, *Fusarium fujikuroi*, in the absence or presence of *S. bellus* (SB) or *S. saprophyticus* (SS). Control: Soil without *Fusarium* inoculation. Values represent means of five replicates after 5 weeks of cultivation and errors bars mark standard deviation. Different lowercase letters above bars shows significant differences between treatments within $p \leq 0.05$.

Figure S1. Sugar beet seedlings at 60d post seeding grown under different nutrient sources and in the absence or presence of rhizosphere bacteria. Sugar beet was grown in soil with no nutrient addition (N0K0P0), orthoclase (OT), rock phosphate (RP) and both nutrient sources (RP+OT), in the absence or presence of *Streptomyces bellus* (SB) or S. *saprophyticus* (SS).

Figure S2. Suppression of sugar beet root infection with *Fusarium* by the streptomycetes. Shoot length and dry weight of sugar beet were measured. Treatments include *Streptomyces* inoculated sugar beet seeds, SB: *Streptomyces bellus* (SB) and SS: *S. saprophyticus* (SS), and *Fusarium*-inoculated substrate, CH1: *Fusarium fujikuroi*, CH2: *Fusarium equiseti* and CH3: *Fusarium fujikuroi.* Control treatments are seeds without bacterial and soil without fungal inoculation. Five weeks of cultivation.

Table 1. Treatments tested in the present study. Sugar beet was grown for 60 days in a greenhouse on non-fertilized sugar beet field soil with low available P and K contents. Abbreviations: no nutrient addition ($N_0K_0P_{0)}$, orthoclase (OT), rock phosphate (RP), and both nutrient sources (RP+OT)

Treatment	RP	OT	*Streptomyces bellus* (SB)	*Streptomyces saprophyticus* (SS)
$N_0K_0P_0$	-	-	-	-
$N_0K_0P_0$+SB	-	-	+	-
$N_0K_0P_0$+SS	-	-	-	+
RP	+	-	-	-
RP+SB	+	-	+	-
RP+SS	+	-	-	+
OT	-	+	-	-
OT+SB	-	+	+	-
OT+SS	-	+	-	+
RP+OT	+	+	-	-
RP+OT+SB	+	+	+	-
RP+OT+SS	+	+	-	+

Table 2. Estimation of N (nitrogen), P (phosphate) and K (potassium) concentrations in soil after 60 days of growth of beet sugar as affected by inoculation of *S. bellus* (SB) and *S. saprophyticus* (SS) with or without rock phosphate (RP) and/or orthoclase (OT). Values are mean of three samples ± SD. Different lowercase letters after values shows significant differences between treatments within $p \leq 0.05$.

	Total			**Available**	
	N	**P**	**K**	**P**	**K**
	%	g kg^{-1}			
$N_0P_0K_0$	0.09 ± 0.01e	1.15 ± 0.003e	7.11 ± 0.003c	0.04 ± 0.001b	0.29 ± 0.004c
RP+OT	0.06 ± 0.04 bcde	2.46 ± 0.004k	7.02 ± 0.003b	0.04 ± 0.002d	0.29 ± 0.001c
RP	0.04 ± 0.01 abc	1.50 ± 0.005g	7.45 ± 0.004h	0.04 ± 0.001bc	0.45 ± 0.003h
OT	0.04 ± 0.02 abcd	1.10 ± 0.008d	7.22 ± 0.003e	0.06 ± 0.003ef	0.35 ± 0.004f
$N_0P_0K_0$+SB	0.07 ± 0.03 cde	0.97 ± 0.006a	7.01 ± 0.001a	0.03 ± 0.002ab	0.30 ± 0.003d
RP+OT+SB	0.08 ± 0.01de	2.34 ± 0.002j	7.60 ± 0.003j	0.04 ± 0.002b	0.27 ± 0.005a
RP+SB	0.01 ± 0.03a	1.79 ± 0.005h	7.14 ± 0.004d	0.04 ± 0.002c	0.40 ± 0.001g
OT+SB	0.04 ± 0.02 abc	1.15 ± 0.004e	7.23 ± 0.006e	0.06 ± 0.005f	0.31 ± 0.002e
$N_0P_0K_0$+SS	0.05 ± 0.01 abc	1.01 ± 0.004b	7.26 ± 0.004f	0.04 ± 0.007bc	0.28 ± 0.005b
RP+OT+SS	0.03 ± 0.03ab	2.11 ± 0.005i	7.51 ± 0.005i	0.04 ± 0.003bc	0.28 ± 0.002bc
RP+SS	0.06 ± 0.03 cde	1.18 ± 0.004c	7.45 ± 0.004g	0.05 ± 0.001a	0.30 ± 0.003i
OT+SS	0.08 ± 0.02 bcde	1.04 ± 0.004f	7.38 ± 0.004h	0.03 ± 0.003e	0.52 ± 0.005d

Figure 1

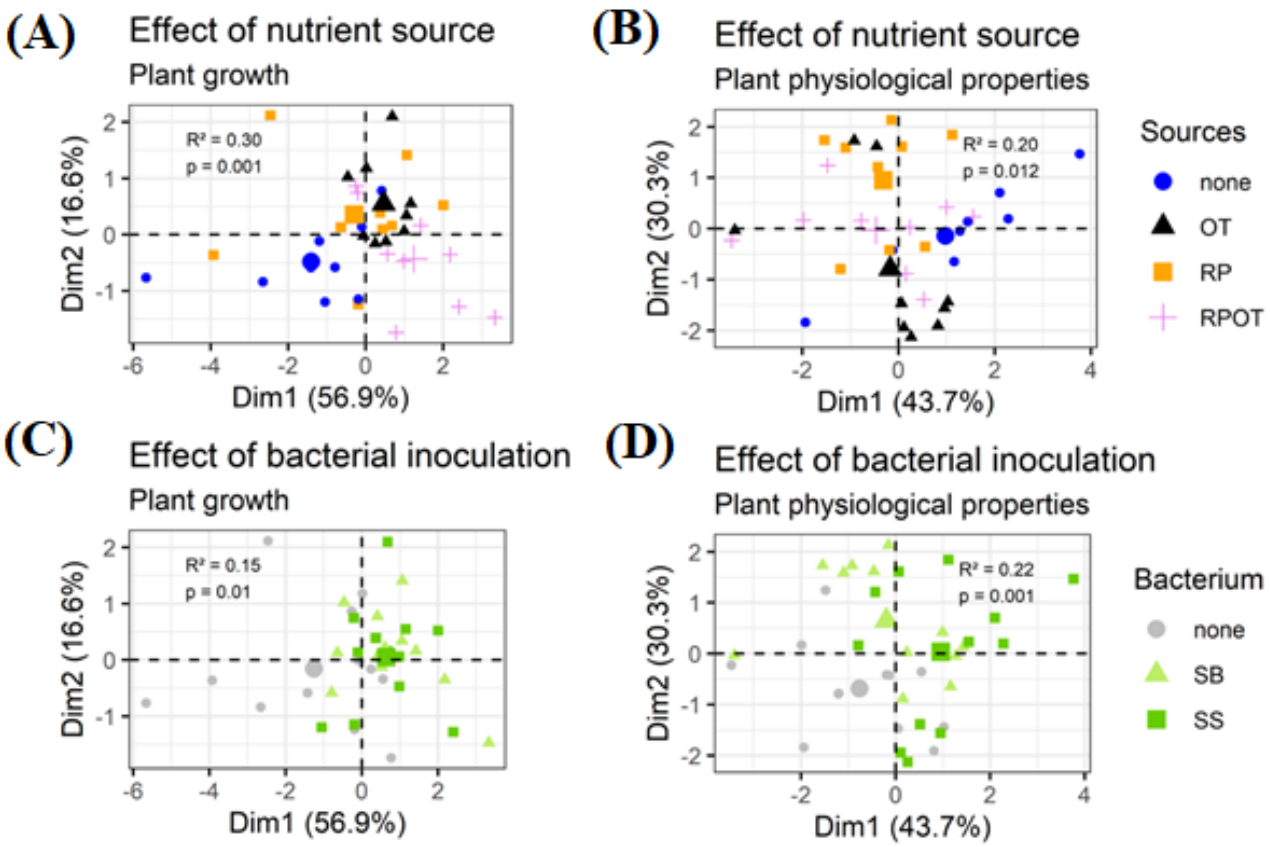

(A)
Effect of nutrient source
Plant growth
R² = 0.30
p = 0.001
Dim2 (16.6%)
Dim1 (56.9%)
(B)
Effect of nutrient source
Plant physiological properties
R² = 0.20
p = 0.012
Dim2 (30.3%)
Dim1 (43.7%)
Sources
none
OT
RP
RPOT
(C)
Effect of bacterial inoculation
Plant growth
R² = 0.15
p = 0.01
Dim2 (16.6%)
Dim1 (56.9%)
(D)
Effect of bacterial inoculation
Plant physiological properties
R² = 0.22
p = 0.001
Dim2 (30.3%)
Dim1 (43.7%)
Bacterium
none
SB
SS

Figure 2

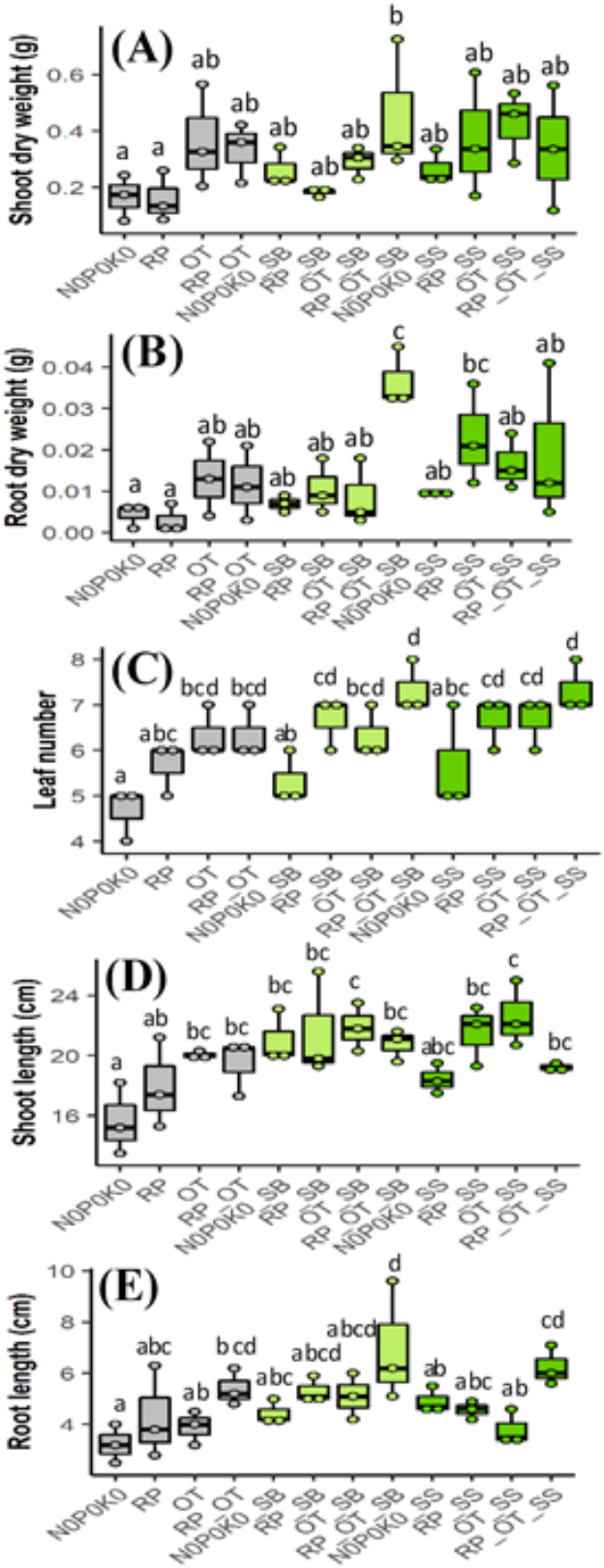

(A)
Shoot dry weight (g)
(B)
Root dry weight (g)
(C)
Leaf number
(D)
Shoot length (cm)
(E)
Root length (cm)
N0P0K0
RP
OT
RP_OT
N0P0K0
SB
RP_SB
OT_SB
RP_OT_SB
N0P0K0
SS
RP_SS
OT_SS
RP_OT_SS

Figure 3

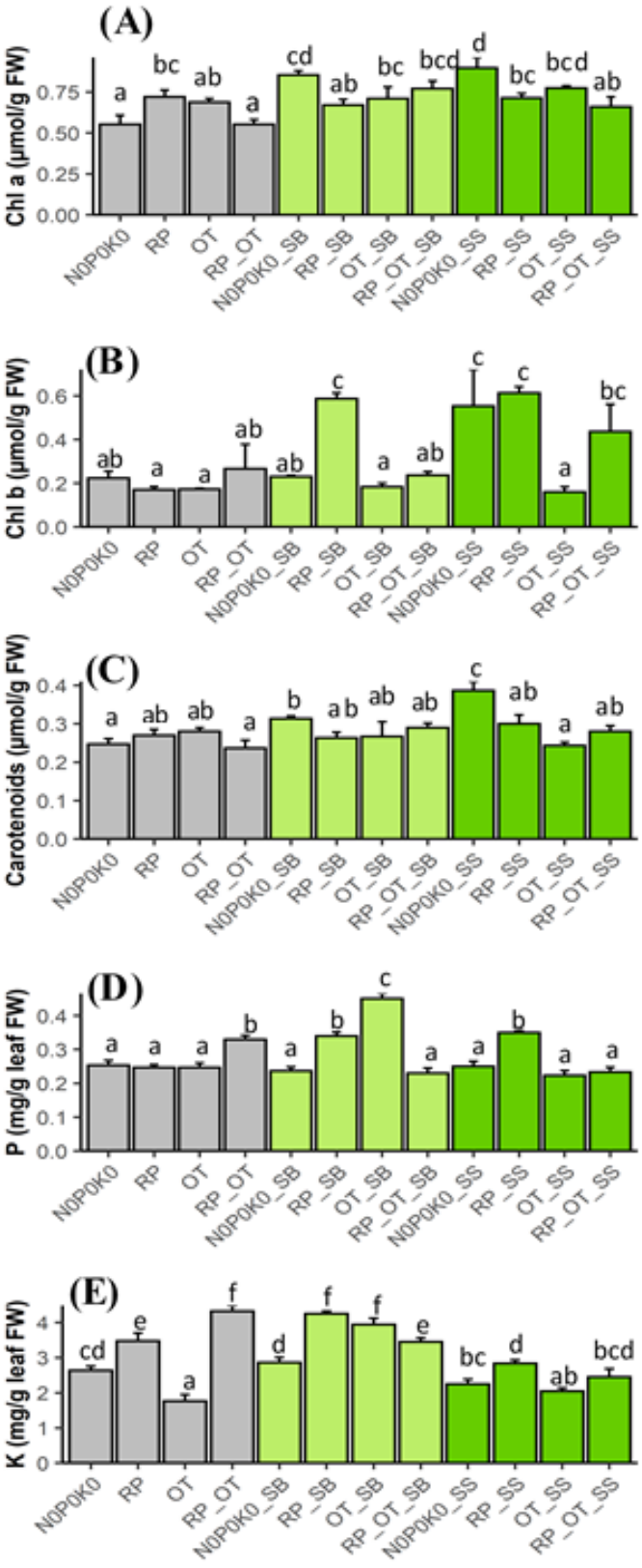

(A)
Chl a (µmol/g FW)
0.00
0.25
0.50
0.75
a bc ab a cd ab bc bcd d bc bcd ab
(B)
Chl b (µmol/g FW)
0.0
0.2
0.4
0.6
ab a a ab ab c a ab c c a bc
(C)
Carotenoids (µmol/g FW)
0.0
0.1
0.2
0.3
0.4
a ab ab a b ab ab ab c ab a ab
(D)
P (mg/g leaf FW)
0.0
0.1
0.2
0.3
0.4
a a a b a b c a a b a a
(E)
K (mg/g leaf FW)
0
1
2
3
4
cd e a f d f f e bc d ab bcd
N0P0K0
RP
OT
RP_OT
N0P0K0_SB
RP_SB
OT_SB
RP_OT_SB
N0P0K0_SS
RP_SS
OT_SS
RP_OT_SS

Figure 4

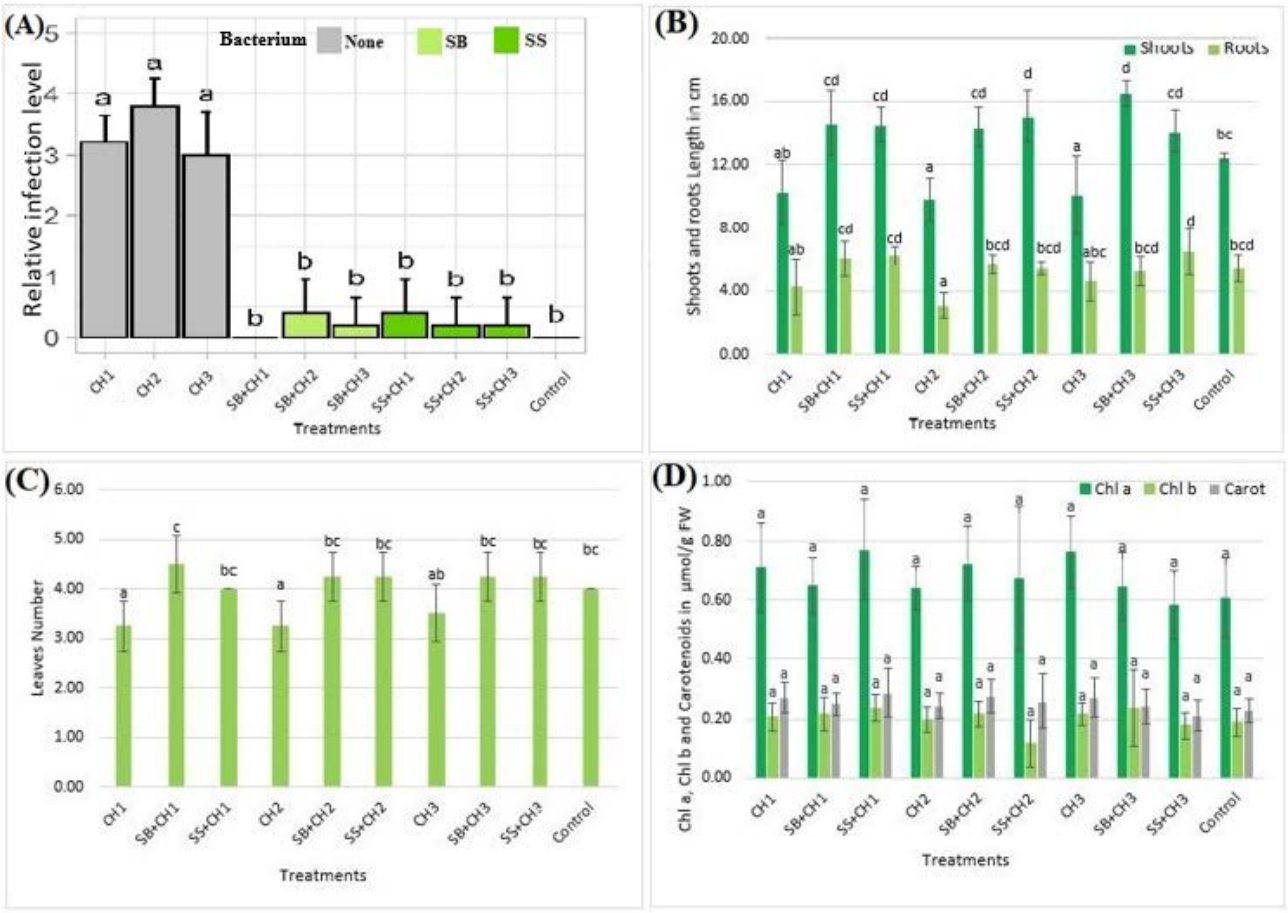
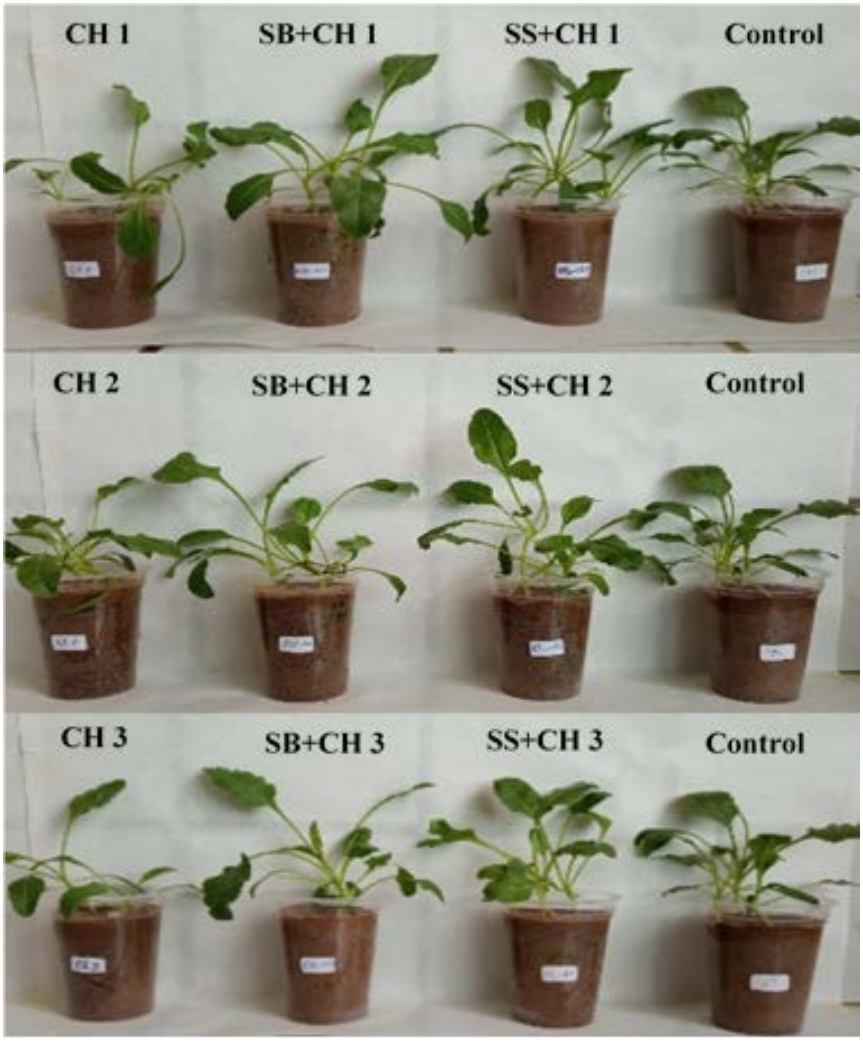

(A)
Bacterium
None
SB
SS
Relative infection level
Treatments
CH1
CH2
CH3
SB+CH1
SB+CH2
SB+CH3
SS+CH1
SS+CH2
SS+CH3
Control
(B)
Shoots
Roots
Shoots and roots Length in cm
Treatments
(C)
Leaves Number
Treatments
(D)
Chl a
Chl b
Carot
Chl a, Chl b and Carotenoids in µmol/g FW
Treatments

Figure S1


40
30
20
10
N₀P₀K₀
RP
RP+SB
RP+ SS
RP+OT+SS
N₀P₀K₀
OT
OT+SB
OT+SS
RP+OT+SB

574 **Figure S2**



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