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Assessment of two endemic rock phosphate solubilizing *Streptomyces* spp. on sugar beet (*Beta vulgaris* L.) growth under field conditions

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Abstract

Biocontrol agents and plant growth-promoting rhizobacteria represent promising tools to improve plant growth and health as environmentally friendly biopesticides and fertilizers. *Streptomyces* bacteria are commonly used for biocontrol, but have been poorly investigated for biofertilizer activity. In this study, we evaluated at field scale the performance of rock phosphate (RP) solubilizing *Streptomyces* spp. (*S. bellus* (SB) and *S. saprophyticus* (SS) in promoting the growth of sugar beet under RP fertilization. Inoculation of seeds with SB stimulated root elongation and inoculation with SS enhanced shoot elongation. At the end of the trial, chlorophyll levels in the leaves were higher with SS. The highest root yield was recorded with SS, and root diameter increased with both bacteria. The levels of soil available phosphorus (P) and potassium (K) increased particularly with SB, but also with SS inoculation. In sum, potassium and phosphate solubilizing and sugar beet growth promoting activities of SB and SS were lower in the field than previously observed in the greenhouse. That the activities were present in the field speaks for improving the formulation and optimizing the application strategy.

Keywords: Actinobacteria, Biofertilizer, Phosphate solubilizing microorganisms, PGPR.

1. Introduction

Most agricultural soils are a reservoir of P, but the phosphorus is not available to plants. It can be trapped by e.g. calcium and magnesium in calcareous soils, immobilized by iron and aluminum in acidic soils, or present in organic forms (C. Xiao et al., 2013). To support the P uptake of plants, readily available chemical fertilizers are mostly applied to agricultural fields, but excessive application of fertilizers can lead to environmental problems such as negative impacts on food chain (Anwar et al., 2016), eutrophication, or soil nutrient imbalance (Naik et al., 2019). Further problems regarding P fertilization are that the global reserves of the raw product of phosphate mines, rock phosphate (RP), are limited and may even be depleted by 2060 (Cordell et al., 2009), but also that a high extent of the applied chemical P fertilizers is converted to insoluble forms that are then not available to plants (Alori et al., 2017; Ben Farhat et al., 2015). Soil fertilization with RP can be considered as an alternative for chemical phosphate fertilizers. RP is namely less expensive than chemical fertilizers and causes less problems to the environment (Xiao et al., 2011). Direct application of RP is unfortunately only effective in acidic soils (Maharana et al., 2020) and its use in alkaline soils is not effective without the assistance of phosphate solubilizing microorganisms (PSM) (Azaroual et al., 2020; Manzoor et al., 2016; Moharana et al., 2018; C. Q. Xiao et al., 2013). Members of Actinobacteria do colonize rhizospheres and plant roots, but they are not traditional PSMs. They are rather known for their abilities to produce antibiotics and serve as biocontrol agents, and their resistance to extreme conditions such as prolonged drought (Ghorbani-Nasrabadi et al., 2013; Olanrewaju and Babalola, 2019; Trujillo et al., 2015). Some members of Actinobacteria do produce siderophores and fix nitrogen (Bousselham et al., 2022; Kakoi et al., 2014; Nafis et al., 2019). Other members of the Phylum stimulate plant growth by synthetizing phytohormones, including cytokinins, gibberellins, and indole-acetic acid (IAA) (Hamdali et al., 2008a; Lehr et al., 2008; Nafis et al., 2019), or by producing 1 aminocyclopropane-1-carboxylic acid deaminase (Gebauer et al., 2021). Some information exists on the performance of PSM Actinobacteria (Hamdali et al., 2021, 2008b, 2008c, 2008a), but only few reports of their performance in the field. Wang et al. (2018) showed that application of *Streptomyces griseoplanus* in combination with a P fertilizer increased soil available P concentration and enhanced plant growth and enhanced grain yield of maize. Also by using maize, Dicko et al. (2018) showed that inoculation with unclassified Actinobacteria led to higher shoot biomass and increased seed yield when compared to the uninoculated control. Furthermore, a field trial using talcum powder formulation of led to higher shoot length and shoot and root weights, total grain yield and weight of grains in rice plants (Tamreihao et al., 2016). But, the potential of Actinobacteria in P solubilization from RP in a field setting, or their performance as a plant growth promoting bacterium with other crops than maize or rice, have not yet been investigated.

To fill this knowledge gap, we investigated the performance of two endemic PSB *Streptomycetes* strains (Aallam et al., 2021a) in a sugar beet field. *Streptomyces bellus* and *S. saprophyticus* strains were previously isolated from sugar beet rhizosphere. They were selected for this field study, since they enhanced sugar beet growth and P acquisition from RP in greenhouse experiments and protected sugar beet from *Fusarium* root rot (Aallam et al., 2021b). To the best of our knowledge, only few studies with biofertilization of sugar beet by bacteria in field scale exist. These include the reports on *Bacillus* (Çakmakçi et al., 2001, 1999; Sahin et al., 2004); *Paenibacillus, Pseudomonas* and *Rhodobacter* (Çakmakçi et al., 2006), but no reports on biofertilization of sugar beet using Actinobacteria exist. This study was carried out in order to evaluate the impact of soil amendment with natural rock phosphate and inoculation

of sugar beet seeds with either of the two *Streptomyces* spp. as phosphate biofertilizer and their effect on sugar beets growth and yield. Based on the performance of the bacteria in the greenhouse studies, their natural phosphate and potassium solubilizing capacity, and the stronger stimulation of sugar beet growth by *S. bellus* than *S. saprophyticus* (Aallam et al., 2022, 2021b, 2021a), this work was based on three hypotheses: i) both isolates stimulate sugar beet shoot and root growth and ii) P and K availability in soil, but iii) *S. bellus* has a stronger impact than *S. saprophyticus.*

2. Materials and Methods

2.1. Description of study and sampling area

The field experiment was carried out in a non-fertilized private farm (32°28'54.9"N, 6°10'50.5"W) located at 25 km south of Beni Mellal region of Morocco in March 2020. The climate in this area is warm Mediterranean with a temperature ranging between 1.1 and 40°C and a mean annual rainfall generally between 350 and 650 mm/year (Barakat et al., 2019).

2.2. Physicochemical analysis of the studied site

The physicochemical properties of the soil are listed in Table S1. According to the FAO-UNESCO (1990) classification, the soil is a calcareous calcisol with a low available P and K concentrations. Various characteristics were measured using standards procedures; particle size distribution (NFX 31-107), pH and electrical conductivity (NF ISO 10390 and NF ISO 11265), $CaCO_3$ (NF EN ISO 10693), organic matter (OM) (NF ISO 14235), exchangeable elements (NFX 31-108 and NF ISO 11263), oligo-elements (Cu, Fe, Mn, Zn) (NFX 31-121) and total N, P and K (Kjeldahl and ICP-OES (Agilent 5110 150 ICP-OES, USA)). In general, the soil was silt sandy with 4.81% OM, 86.00 mg kg^{-1} exchangeable P and 4933.00 mg kg^{-1} total P. 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).

2.3. Inoculum preparation and seeds treatment

Streptomyces bellus MW797036 (SB) and *Streptomyces saprophyticus* MW797316 (SS) isolated from Moroccan sugar beet fields were previously selected for their multiple Potential Plant Growth (PGP) abilities (Aallam et al., 2021a, 2021b). The strains are available at the culture collection "Collections Coordonnées Marocaines de Microorganismes (CCMM)" under the identification numbers CCMM B1328 (SB) and CCMM B1327 (SS). Spores of each strain, 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 (x 4) for 3 days at 28 °C under constant agitation on a rotary shaker (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 40 mL of sterile deionised water. Twenty ml of the mycelial suspension was added to 10 g of wet carboxymethylcellulose (CMC, Merck). This paste was mixed with seeds as indicated later in this section. Four repetitions were conducted for each treatment and for each plot of the experiments.

Sugar beet (*Beta vulgaris* L.) seeds were harvested in October 2019, and obtained from COSUMAR, the major Moroccan sugar manufacturer (www.cosumar.co.ma). Surface sterilization of the 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. Each seed was coated by a thin layer of wet CMC, containing 10^6 colony forming units bacteria, as determined by plating on Bennett agar.

2.4. Sugar beet field trial

The sugar beet field trial was conducted in a well-irrigated area during six consecutive months. Mineral and biofertilizer were applied in different treatments in a randomized complete block design (RCBD) on a plot size of 2 m x 10 m with four sub-plots with 6 m^2 (3m x 2m) in each area and spacing with 0.5 m x 2 m with four replicates (Fig. S1). The distance between plants and rows were maintained at 20 cm and 40 cm, respectively. This measured area contains 180 plants in each plot indicated in the supplementary material (Fig. S1). After sowing the plots were irrigated immediately. Furthermore, all others practice such as irrigation, fertilization and herb control were made in the same way of farmers in the region. Flood irrigation was applied twice a week and weed was controlled mechanically by workers. After 30 days from sowing, plants were thinned to only one plant/hill.

The five treatments used in this study were:

- Treatment 1: Uninoculated Seeds in soil amended with NPK (nitrogen, phosphorus and potassium) as positive control (NPK)
- Treatment 2: Uninoculated Seeds in soil without NPK as negative control ($N_0P_0K_0$)
- Treatment 3: Coated sugar beet seeds with *S. saprophyticus* strain in soil amended with natural RP as sources of phosphorus, urea as source of nitrogen and potassium sulphate as source of potassium (SS)
- Treatment 4: Coated sugar beet seeds with *S. bellus* strain in soil amended with natural RP as sources of phosphorus, urea as source of nitrogen and potassium sulphate as source of potassium (SB)
- Treatment 5: Uninoculated seeds in soil amended with natural RP as sources of phosphorus, urea as source of nitrogen and potassium sulphate as source of potassium (RP)

For NPK fertilization, Nitrogen was used in the form of urea (33% N) at rate of 300 kg ha^{-1}, two times at 6 leaf stage (2 months) and after 4 months of sowing. Phosphorus and potassium were applied in the form of phosphoric anhydride (23% P_2O_5) at rate 250 kg ha^{-1} and in the form of potassium sulphate (50% K_2O) at rate of 250 kg ha^{-1} respectively, the rock phosphate was applied at rat of 30kg/ha. One time at planting time.

2.5. Plant sampling and parameters analysis

Agronomic parameters such as shoot length (from the plant base to the top of the first fully developed leaf), root length, dry matter and chlorophyll concentration as a measure of photosynthetic potential were measured at 30, 60, 90, 120, 150 and 180 days after sowing by sampling four plants per plot. After harvesting, the parts of the sugar beet plants were dried in the oven at 70 °C to obtain a constant weight. The samples were dried, ground and sieved.

At maturity (180 days from planting), 1 m x 1 m in area was harvested from each sub-plot to measure the following parameters: root yield (t ha^{-1}), shoot yield (t ha^{-1}), sucrose concentration (%) by handheld refractometer (Atago Co, Japan), and root diameter. Then, plant material (one plant/sub-plot) was dried to determine the accumulation of total nitrogen, phosphorus and potassium in roots and shoots. Soil samples from each sub-plot were collected and analyzed for N, P and K determination. Total nitrogen was analyzed using Kjeldahl method, available P according to NF ISO 11263 and available K according to NFX 31-108.

2.6. Statistical analysis

Root and shoot length and dry weight of roots and shoots were subjected to two-way analysis of variance (ANOVA) to determine the effect of both treatments and time. First, the data were transformed to obtain normal distribution. Then, a one-way analysis was performed to compare the effect of different treatments on sucrose, shoot and root yield and element concentrations in soil, root and shoot at end point. Significant differences between means were compared using Duncan's tests at a 5%. All data were treated using SPSS software 20.0 package for Windows.

3. Results

3.1. Effect of inoculation with Streptomyces bellus (SB) or S. saprophyticus (SS) on sugar beet growth

Sugar beet growth was followed for 6 months (M1-M6) with no nutrient addition ($N_0P_0K_0$), recommended nutrients (NPK), rock phosphate (RP), and RP with seed inoculation with SB or SS (Fig. S1). The analysis of results by the two-way ANOVA indicated significant effect on sugar beet shoot and root lengths by treatment and time ($p < 0.05$). Shoot length was increased by SS in M5 and root length by M4 and M5 by SB. As expected, the plant growth parameters were at their lowest levels in the non-supplemented soil $N_0P_0K_0$, and compared to that, higher and comparable between RP and NPK fertilization (Fig. 1). Shoot and root biomass was largely unaffected from the treatments. During M4, SB had a negative influence on shoot biomass, and root biomass was at its highest with $N_0P_0K_0$ (Fig. 2).

3.2. Effects of Streptomyces spp. on plant physiological parameters

The influence of *Streptomyces* spp. on chlorophyll a, chlorophyll b and carotenoid concentrations are presented in Fig. 3. Higher chlorophyll a levels were recorded with SS during M3, and chlorophyll b levels again with SS during M6. By contrast, carotenoid levels were not affected by the bacteria. The effects of fertilizer applications were context dependent. For instance, higher chlorophyll a and carotenoid levels were recorded for NPK than RP during M5, but not during other months.

3.3. Effects of the Streptomyces spp. on N, P and K concentrations of sugar beet plants at harvest

Shoot N concentration (Fig. 4) was highest in SS. Surprisingly, the levels of N in shoots were at their lowest with SB. Root N concentrations were lower with SB or SS, close to the values in $N_0P_0K_0$. The two bacteria did not affect shoot or root P concentrations (Fig. 4). In shoots, lowest P concentrations were recorded in $N_0P_0K_0$ and NPK, and in roots, the only significant difference was between SB (higher) and $N_0P_0K_0$ (lower). The bacteria had contrasting effects on shoot and root K concentrations (Fig. 4). For shoots, K concentrations were lower with SB or SS than in RP. Apart from that, very low values were detected for $N_0P_0K_0$ and NPK. For roots, K concentration increased with SS.

3.4. Effect of Streptomyces spp. inoculation on sugar beet yield at harvest

Shoot yield at harvest was not affected by the streptomycetes, and it was comparable between RP and NPK (Fig. 5). Root yield increased with SS and reached a comparable value with SS to that in NPK (Fig. 5). Root diameter increased by the inoculation with SB and with SS, and the values were comparable to that in NPK. The growth parameters at harvest were consistently lower in $N_0P_0K_0$ than in other treatments.

3.5. Effect of Streptomyces spp. on sugar beet sucrose concentration

The only significant differences between the sugar beet sucrose concentrations were the higher value with SB than with $N_0P_0K_0$ or NPK (Fig. 6). Treatment with SB showed the highest sucrose concentration of 16.6 % of fresh weight, and the values for SS and RP were similar, 15.7 % and 15.6 %, respectively.

3.6. Effect of Streptomyces spp. on N, P and K concentrations of the soil

At harvest, we also investigated how the bacterial inoculation affected soil total N, and available P and K levels (Table 1). Both *S. bellus* and *S. saprophyticus* increased the total concentration of available P and K in soil, and the effect of SB was higher than that of SS. The concentrations of available P and K were at their lowest in $N_0P_0K_0$, available P was lower in RP than in NPK, and available K *vice versa*.

4. Discussion

Based on the performance of two endemic Streptomyces isolates in vitro and by greenhouse studies, showing mineral phosphate solubilizing, sugar beet growth promoting and disease suppressing activities (Aallam et al. 2021a, b), we conducted a field experiment to see if this benefit is maintained outside the laboratory. Results showed, that root yield increased with *S. saprophyticus* (SS), reaching a comparable value with SS to that in NPK fertilization. Furthermore, both SS and SB treatments led to an increased sugar beet root diameter. This is to our knowledge the first field scale report on a PGPR *Streptomyces* strain promoting sugar beet yield, and it supports the findings with other plant species suggesting that plant associated Actinobacteria provide plant beneficial traits (Goudjal et al., 2013; Nafis et al., 2019; Olanrewaju and Babalola, 2019; Worsley et al., 2020). The concentrations of available P and K in soil were increased by the treatment with SB and SS, supporting the view that searching for rhizosphere microorganisms that solubilize minerals *in vitro* and *in vivo* is a promising screening strategy to obtain PGPR (Gondal et al. 2021; Miransari 2011; Wang et al. 2021). Our earlier *in vitro* and greenhouse experiments indicated that a treatment with SB has a stronger positive influence on P and K availability in soil and uptake by plants than with SS, and that SB has a higher capacity to produce indole acetic acid (IAA) and stimulate sugar beet growth than SS (Aallam et al., 2021a, 2021b). Whereas the positive influence on available P and K in the soil was confirmed in the field, only SS significantly improved sugar beet production. These observations provide further evidence that although results from in vitro assays and greenhouse experiments can be promising, they have to be further elaborated in the field scale (Aallam et al., 2022).

The inoculation of sugar beet seeds had only a minor impact on sugar beet seedlings: increased shoot length by SS during the fifth month and increased root length by SB during the fourth and fifth month (Fig. 1). This observation supports only in part our first hypothesis, that stated a higher growth rate of plants with bacterial inoculation. The positive influence on roots and shoot elongation is in agreement with the earlier results of Mohamed and El Sebai (2019) using *Burkholderia* sp., *Bacillus* sp. and *Acinetobacter* sp. isolates with sugar beet. Apart from

the positive influence of SS on sugar beet yield, shoot or root biomass were not affected by SB or SS. In contrast, numerous works have reported that microorganisms stimulate plant growth when RP is added to the soil. Examples include wheat inoculated with *Enterobacter aerogenes*, *Bacillus megaterium* and *B. safensis* (Mukhtar et al., 2017), maize and wheat with *Pantoea cypripedii* and *Pseudomonas plecoglossicida* (Kaur and Reddy, 2015), tomato with *Paenibacillus polymyxa* and *B. megaterium* (Elyazied and Abou-aly, 2011), but also sunchoke with *Klebsiella variicola* strain also increased its dry matter after application of RP as insoluble P source under field conditions (Nacoon et al., 2021): in the presence of RP, a seed inoculation with these bacteria promoted root and shoot growth under field conditions. What was not evaluated in this study, was the contribution of P release from organic sources. Streptomycetes are also known for their role in mineral acquisition through phytase activity (Puppala et al., 2019) and phytase production by PSMs is considered to be an important factor in the biofertilization process (Rasul et al., 2021). The chlorophyll concentration of sugar beet leaves, indicative of improved nitrogen acquisition, was enhanced only by SS and at a certain stage of growth. Higher chlorophyll level was recorded during the third month and chlorophyll b levels at harvest. These results are consistent in part with those observed in sugarcane by Chauhan et al. (2013), who showed that the inoculation by *Gluconacetobacter* and *Azospirillum* spp. under field conditions increased chlorophyll concentrations of sugarcane leaves.

Availability of P and K in soil was increased by both bacteria, especially by SB (Fig. 7). This result is in line with the second hypothesis, which suggested better soil availability of P and K in the presence of the bacteria, as well as the third hypothesis, stating that SB has a stronger effect than SS. One would expect that the mobilization of P and K by these strains leads, in the absence of increased plant biomass, to higher P and K concentrations in the plants, but this was not observed. Instead, treatment by the two bacteria had no effect on P concentrations, induced lower shoot K concentrations, and only in the roots with SS inoculation an increased K concentration. The negligible effect on P uptake may be due to the calcareous nature of the field soil, alike what was reported by Shaheen et al. (2021) where P is strongly associated to the mineral under a higher pH: surface adsorption and precipitation lower the P availability and mobility in calcareous soils (Pizzeghello et al., 2011), and even by stimulating soil P and K availability, the influence of the bacteria may not have been sufficient for stimulating plant mineral nutrient uptake. In support to our results, *Pseudomonas fluorescens* and *P. chlororaphis* treatments of sugar beet grown on calcareous soil did not increase plant K levels (Sacristán-Pérez-Minayo et al., 2020). Our earlier greenhouse study indicated that SB and SS stimulate plant P and K levels especially when both RP and K mineral are applied (Aallam et al., 2022, 2021a). This raises the question whether RP amendment should be coupled with the application of orthoclase or another K mineral, to support the functionality of SB and SS.

Sugar beet yield was enhanced by SS and sugar beet diameter by both bacteria (Fig. 3) but sugar beet sucrose content did not change significantly (Fig. 4). By contrast, treatments by N-fixing and/or P-solubilizing *Paenibacillus polymyxa*, *Pseudomonas putida* and *Rhodobacter capsulatus* (Çakmakçi et al., 2006, 2001) in field conditions stimulated sugar beet sucrose concentrations, as well as the inoculation of sugarcane with the PSB *B. megatherium* (Sundara et al., 2002). In addition, it was found that 50% of the costly super phosphate could be replaced by RP when applied in conjunction with the PSB *B. megatherium*. The effects of SB and SS on sugar beet N concentration were mostly negative (Fig. 7). This is unfortunate, since the elevation in N levels stimulates the growth of plants and the

uptake and utilization of P and K (Leilah and Khan, 2021). For instance, the inoculation of *Pseudomonas* spp. with RP increased not only P but also N concentrations of shoots and roots of wheat plants, suggesting a synergistic link between improved N and P nutrition (Elhaissoufi et al., 2020). That suggests that a combination of N fixing or N uptake facilitating bacteria with SB and SS should be tested in the field.

In greenhouse studies, we observed biocontrol activity of *S. bellus* and *S. saprophyticus* against *Fusarium* spp., causal agents of rot root disease in sugar beet plants (Aallam et al., 2022). Since the control plants of this field experiment were not affected by root pathogens, we could not estimate the biocontrol potential in the field. This would be important, as successful field trials with biocontrol Actinobacteria have been rare (Ebrahimi-Zarandi et al., 2022). Applications of phosphate solubilizing bacteria can influence the bacterial community of inoculated soil, which may affect the response of the plants to the inoculant. For instance, Chouyia et al. (2020) showed that inoculation with *S. roseocinereus* TOR3209 stimulated the density of total prokaryotes and total Actinobacteria in the rhizosphere. TOR3209 inoculation led to higher tomato biomass and fruit yield but also increased the relative abundances of families *Flavobacteriaceae, Sphingobacteriaceae, Polyangiaceae and Enterobacteriaceae* in tomato rhizosphere. In a pot experiment with TOR3209 and a *Bacillus velezensis* isolate from tomato rhizosphere the strains jointly promoted tomato production, but during this process, the abundance of inoculated TOR3209 strongly decreased in the rhizosphere (Hu et al., 2020).

5. Conclusions

In conclusion, *Streptomyces bellus* and *S. saprophyticus* strains promoted P and K availability in the soil and *S. saprophyticus* sugar beet yield. Based on our field results, we anticipate that *S. saprophyticus* could be used as an ecological and sustainable bio-fertilizer for sugar beet agriculture. According to literature Gram positive bacteria are adequate for coating the seeds due to their thick peptidoglycan cell wall. Another field site and at least two seasons need to be investigated to test how robust the isolates are in the field. Finally, quantitation of the PSMs during the field experiment and evaluating the changes in rhizosphere community composition and suppression of soil borne pathogens would give valuable details on the mechanisms behind sugar beet growth promotion.

Disclosure statement.

The authors report there are no competing interests to declare.

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Table 1. Soil N, P and K concentrations after 6 months sugar beet growth in field conditions. RP: sugar beet with rock phosphate; SB: sugar beet seeds inoculated by *S. bellus* (SB) with RP; SS: sugar beet seeds inoculated with *S. saprophyticus* (SS) with RP, $N_0P_0K_0$: negative control; NPK: positive control. Values are mean of three samples ± SD.

	Total	**Available**	
	N	**P**	**K**
	%	mg kg^{-1}	
SB	0.15 ± 0.02a	89.33 ± 3.05a	586.33 ± 2.08a
SS	0.11 ± 0.02b	79.17 ± 0.76b	391.00 ± 2.65b
RP	0.15 ± 0.01a	61.33 ± 1.16d	384.67 ± 1.53c
$N_0P_0K_0$	0.06 ± 0.01c	55.00 ± 2.65e	295.67 ± 1.53e
NPK	0.15 ± 0.02a	74.67 ± 2.08c	358.33 ± 2.52d

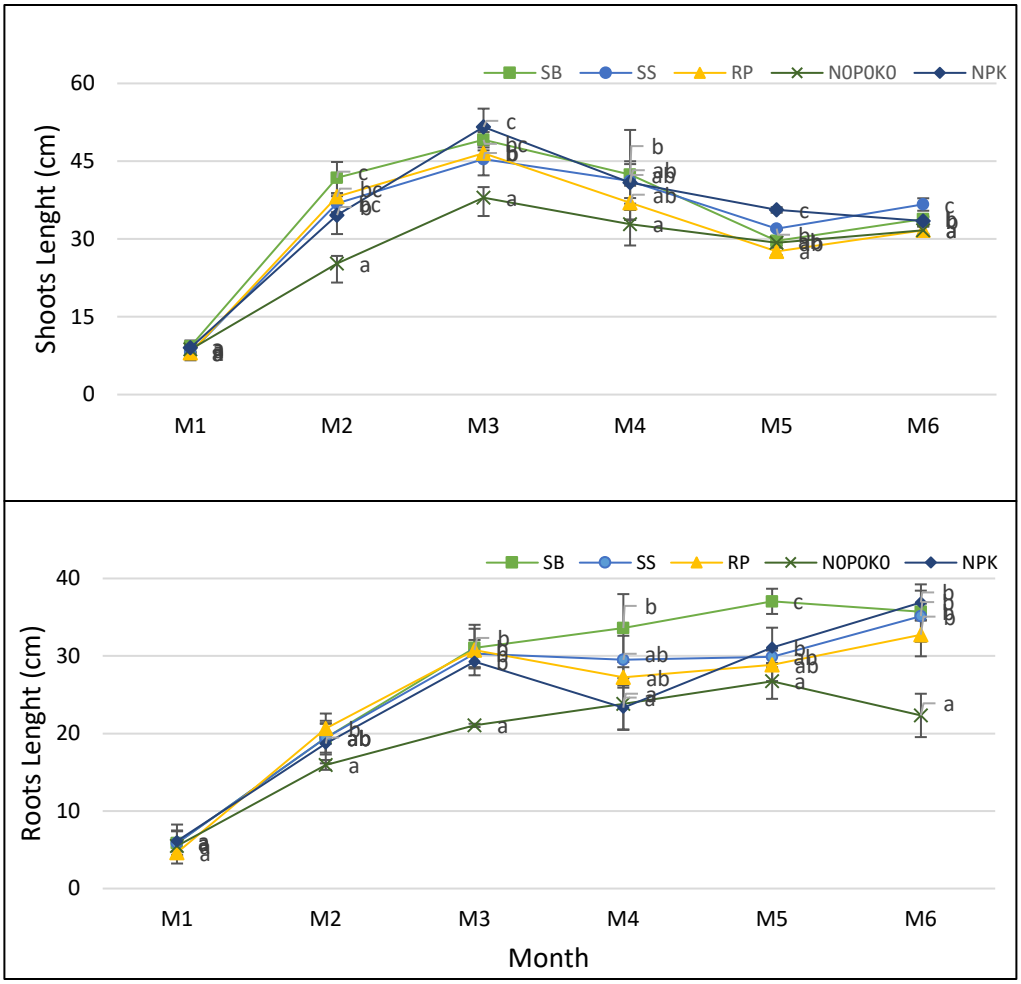
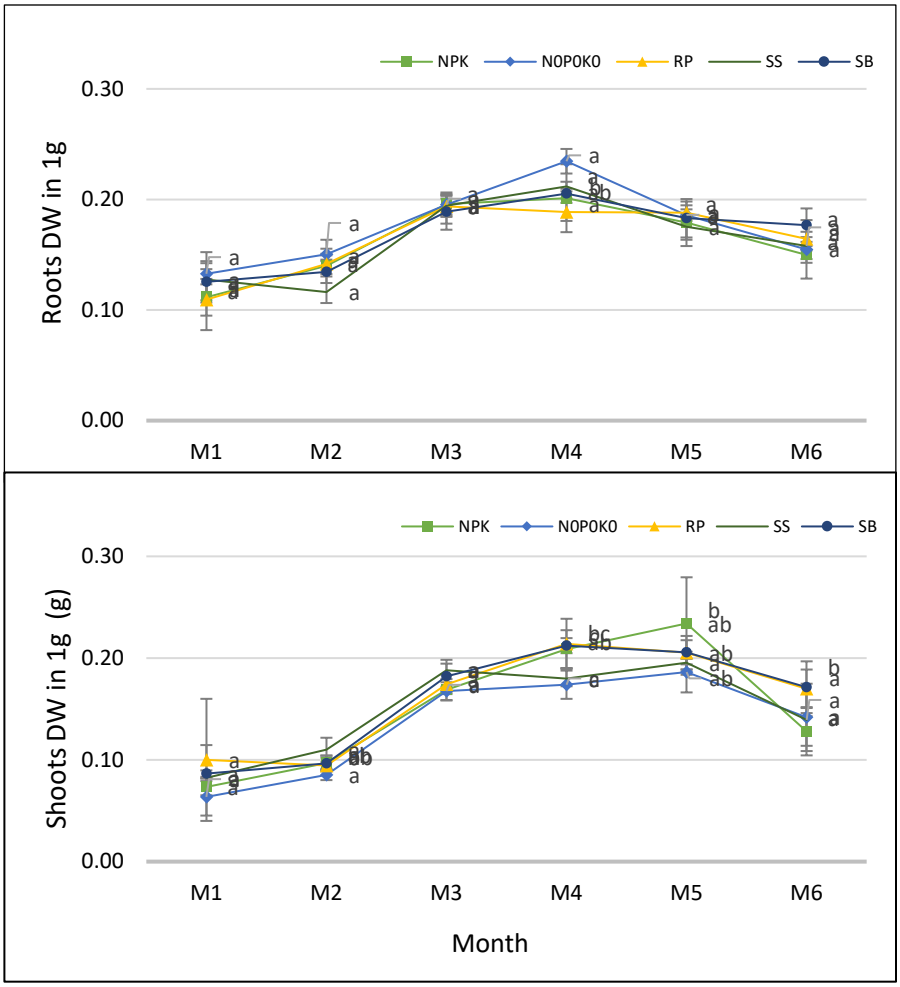


Fig. 1 Shoot and root lengths (cm) of sugar sugar beet during 6 months. SB: Soil inoculated with *Streptomyces bellus* in the presence of RP; SS: Soil inoculated with *S. saprophyticus* (SS) in the presence of RP; $N_0P_0K_0$: negative control; NPK: positive control and RP: Rock phosphate without inoculation. Values are means of four replicates and errors bars represent standard deviation. Different lowercase letters above bars shows significant differences between treatments according to ANOVA ($p \leq 0.05$). M1-M6, the month of measurement. Note that in each month, 4 sugar beet plants were harvested and used for the analyses.



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489 **Fig. 2** Sugar beet shoot and root biomass (dry weight) during 6 months. SB: Soil inoculated with *Streptomyces bellus*
490 in the presence of RP; SS: Soil inoculated with *S. saprophyticus* (SS) in the presence of RP; $N_0P_0K_0$: negative control;
491 NPK: positive control and RP: Rock phosphate without inoculation. Values are means of four replicates and errors
492 bars represent standard deviation. Different lowercase letters above bars shows significant differences between
493 treatments according to ANOVA ($p \leq 0.05$). M1-M6, the month of measurement. Note that in each month, 4 sugar
494 beet plants were harvested and used for the analyses.

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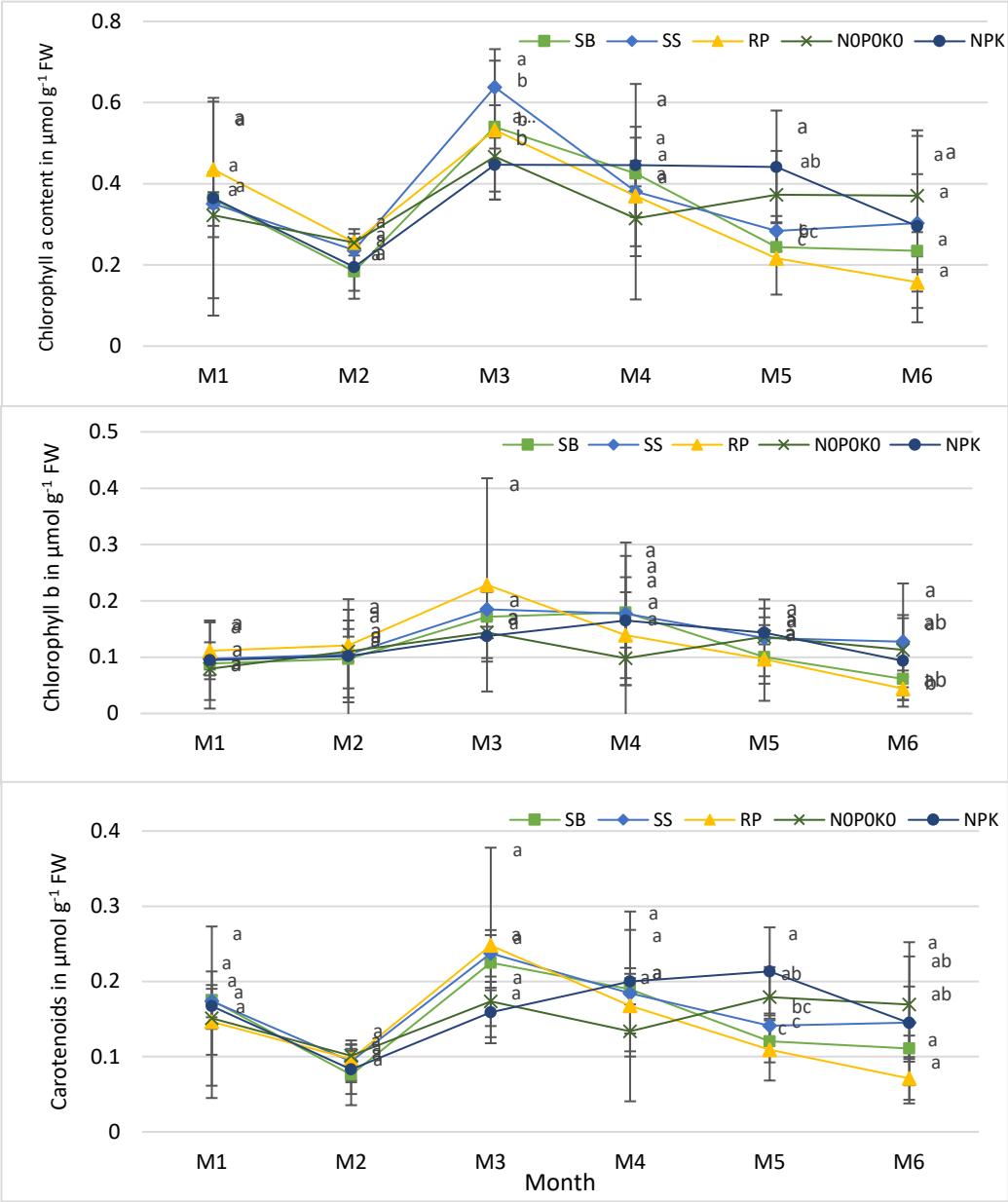


Fig. 3 Effects of *Streptomyces* inoculation on plant chlorophyll a, chlorophyll b and relative carotenoid content. SB: Soil inoculated with *Streptomyces bellus* in the presence of RP; SS: Soil inoculated with *S. saprophyticus* (SS) in the presence of RP; $N_0P_0K_0$: negative control; NPK: positive control and RP: Rock phosphate without inoculation. Values are mean of four replicates and error bars represent standard deviation. Different letters above the bars show significant differences between treatments within $p \leq 0.05$. M1-M6, the month of measurement. Note that in each month, 4 sugar beet plants were harvested and used for the analyses.

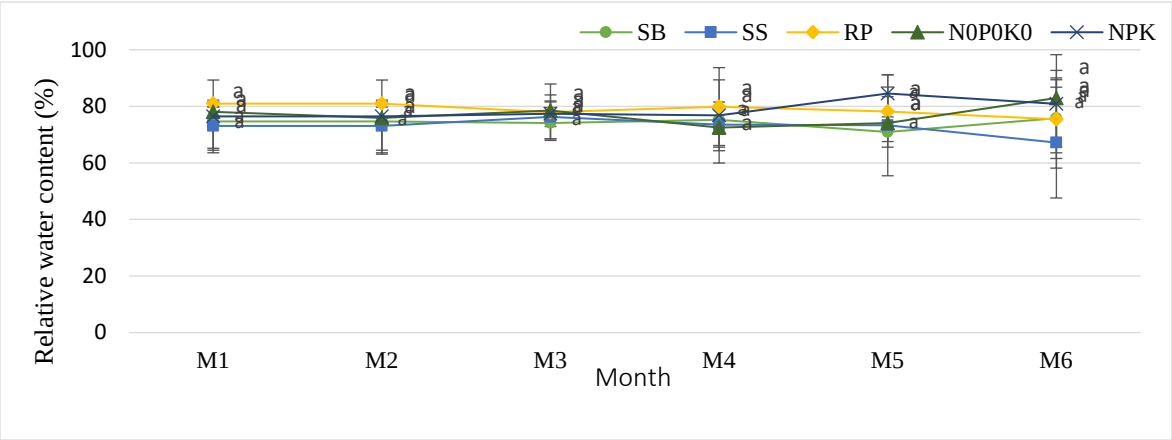
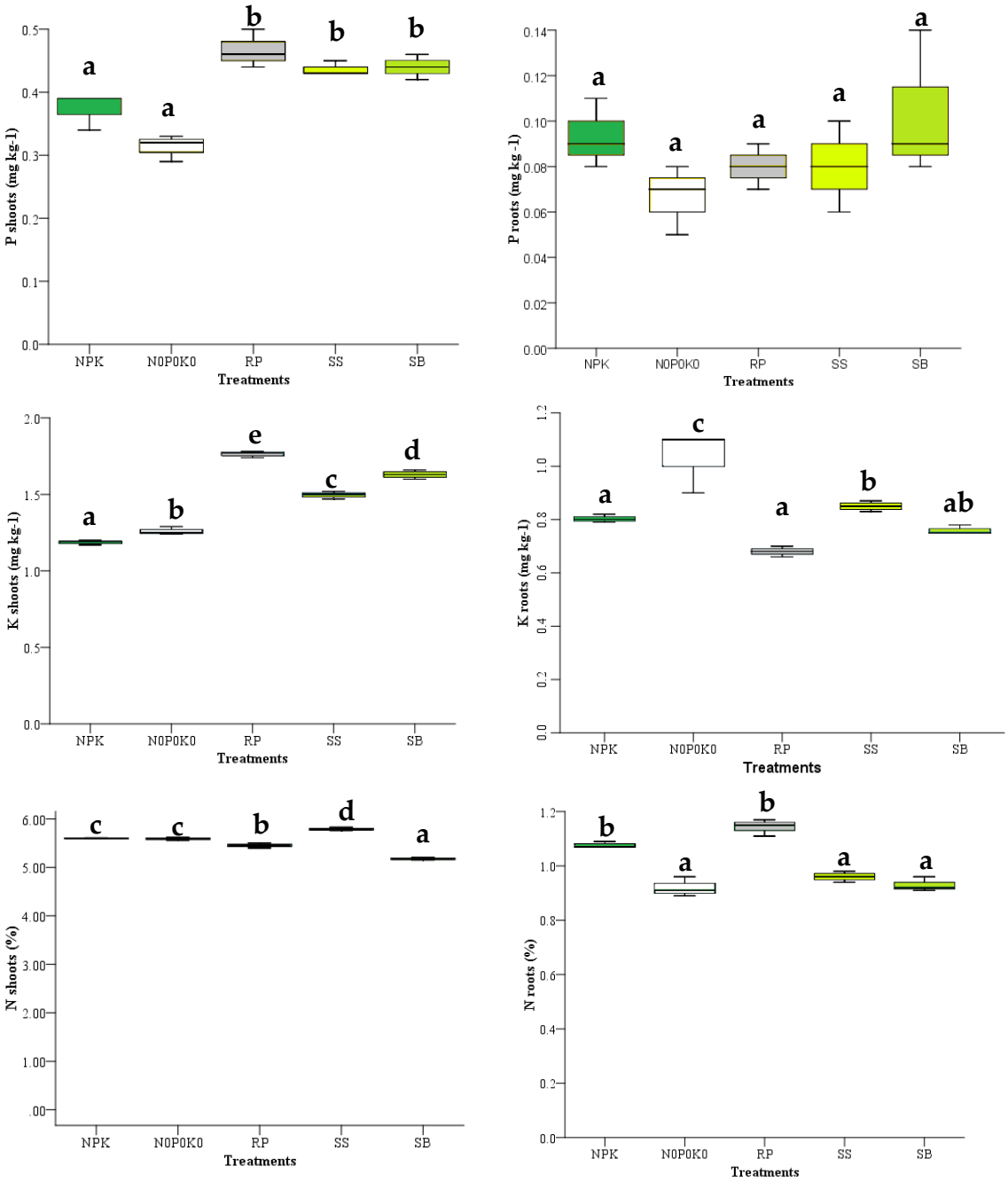


Fig. 4 Effects of *Streptomyces* inoculation on leaf relative water content (RWC). SB: Soil inoculated with *Streptomyces bellus* in the presence of RP; SS: Soil inoculated with *S. saprophyticus* (SS) in the presence of RP; $N_0P_0K_0$: negative control; NPK: positive control and RP: Rock phosphate without inoculation. Values are mean of four replicates and error bars represent standard deviation. M1-M6, the month of measurement. Note that in each month, 4 sugar beet plants were harvested and used for the analyses.



513

514 **Fig. 5** Estimation of N, P and K in sugar beet shoots and roots. Inoculations with *S. bellus* (SB) or *S. saprophyticus*
515 (SS) in presence of rock phosphate (RP). $N_0P_0K_0$: negative control; NPK: positive control and RP: Rock phosphate
516 without inoculation. Boxplots represent four replicates after 6 months of cultivation (equls to M6 of earlier figures).
517 Different lowercase letters above bars shows significant differences between treatments within $p \leq 0.05$

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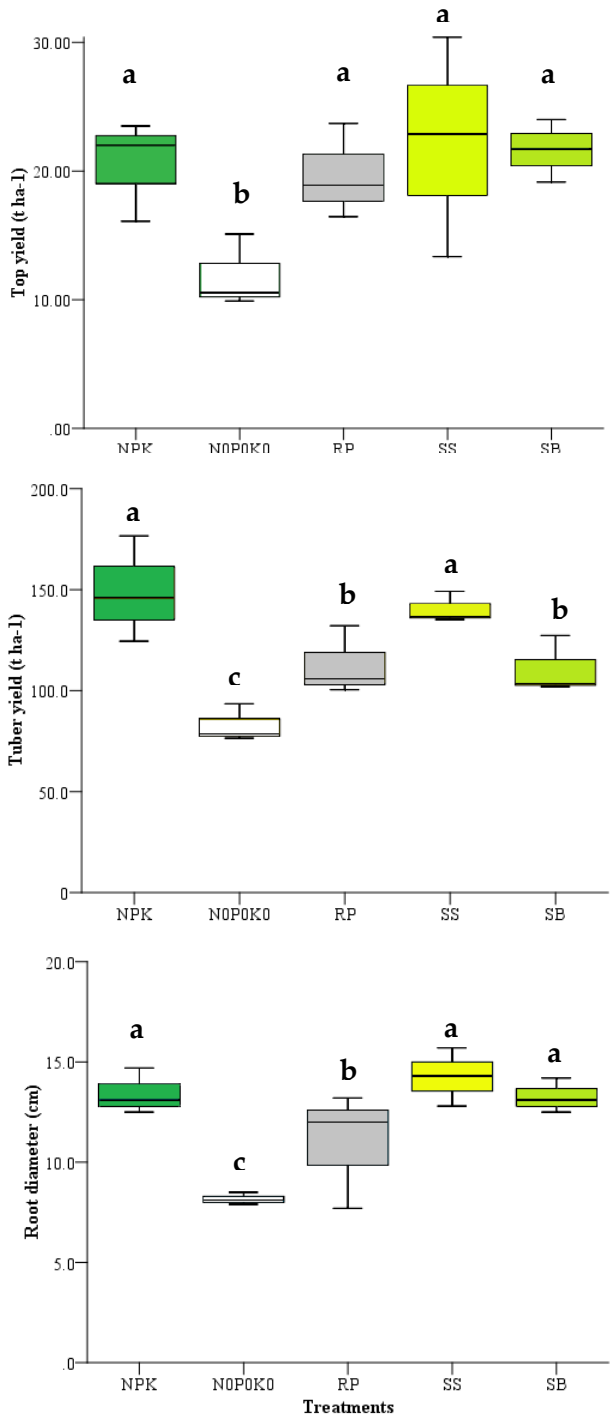

Fig. 6 Growth parameters of sugar beet plants. Shoots yield, Roots yield and root diameter of sugar beet plants without bacterial inoculation or with *S. bellus* (SB) or *S. saprophyticus* (SS), and in the presence of rock phosphate (RP) as P source. $N_0P_0K_0$: negative control; NPK: positive control and RP: Rock phosphate without inoculation. Boxplots represent four replicates after 6 months of cultivation. Different lowercase letters above bars shows significant differences between treatments within $p \leq 0.05$.

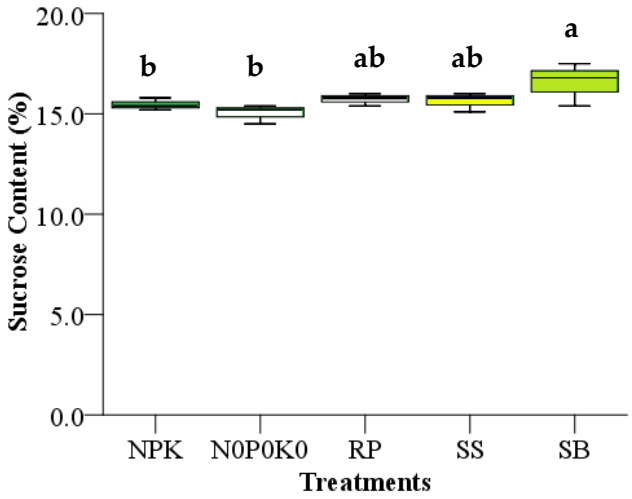

Fig. 7 Sucrose concentration in sugar beet plants. Inoculations with *S. bellus* (SB) or *S. saprophyticus* (SS) in the presence of rock phosphate (RP). $N_0P_0K_0$: negative control; NPK: positive control; RP: Rock phosphate without inoculation. Boxplots represent four replicates after 6 months of cultivation. Different lowercase letters above bars shows significant differences between treatments within $p \leq 0.05$