

ORIGINAL ARTICLE

***Streptomyces* sp. mitigates abiotic stress response and promotes plant growth**

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Abstract

Coexisting microorganisms are abundant in nature. Plant growth promoting rhizobacteria (PGPR) is a group of beneficial microorganism living around the roots of plants which are able to confer beneficial effects on plant growth. *Streptomyces* sp. is a gram-positive bacteria as PGPR that can promote plant growth and enhance tolerance in adverse environment. This research was aimed to study the effects of plant growth promotion and stress tolerance of *Streptomyces* sp. in *Arabidopsis* and *Brassica* sp. The amount of indole-acetic acid (IAA) and phosphate solubility were assessed from isolated bacterial. Plant growth promotion was examined in 10-days old seedling with three independent experiments. Our results showed that *Streptomyces* sp. produced moderate levels of IAA and it was able to solubilize phosphate. Inoculation of *Streptomyces* sp. enhanced lateral root number, fresh weight and chlorophyll content in *Arabidopsis thaliana*. Moreover, the inoculation of *Streptomyces* sp. significantly increased vegetative growth on *Arabidopsis* and *Brassica* sp. by producing higher fresh weight and chlorophyll content. *Streptomyces* sp. also enhanced tolerance to abiotic stress in *Arabidopsis* and *Brassica* sp. by increasing fresh weight under condition of salt and heat stress. Under salt stress, inoculation of *Streptomyces* sp. in *Arabidopsis* induced activity of catalase enzyme and decreased hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) production. In the molecular levels, *Streptomyces* sp. induced protein accumulations in *Arabidopsis* including nitrogen assimilation (GS1), carbohydrate metabolism (cFBPase), and the light-harvesting chlorophyll (Lhcb1) protein.

Keywords: indole-3-acetic acid (IAA), catalase enzyme, solubilize phosphate, malondialdehyde (MDA) production, *Streptomyces* sp.

Introduction

Agriculture has strongly felt the effects of climate change occurring throughout the world. Climate change threatens food security throughout the developing countries (Mosttafiz *et al.* 2012). Global climate change accelerates the concurrence of a variety of abiotic (e.g., drought, salinity, heavy metals, and extreme temperatures) and biotic stresses (e.g., phytopathogens). The dependence of agriculture on chemical fertilizers and pesticides has also resulted in adverse impacts on the environment (Ma *et al.* 2019). Nowadays, plant growth promoting microorganisms (PGPM) are receiving increasing attention by agronomists and environmentalists as candidates to develop an effective,

eco-friendly, and sustainable alternative to conventional agricultural (e.g., chemical fertilizers and pesticides) and remediation (e.g., chelate-enhanced phytoremediation) methods employed to deal with these climate-change-induced stresses (Ma *et al.* 2011; Ma *et al.* 2016). Using microorganisms as biocontrol and organic fertilizers has become an alternative way to reduce the adverse effects of chemicals used in agriculture (Bhardwaj *et al.* 2014).

Plant growth promoting rhizobacteria (PGPR) is a group of beneficial microorganisms living around the roots of plants which are able to confer beneficial effects on plant growth without causing damage to the

host plant (Ahemad and Kibret 2014). *Streptomyces* sp. is a gram-positive bacteria that is known to have a high ability of producing numerous antimicrobial compounds for functioning as a biocontrol agent (Evangelista and Martínez 2013). Besides being a biocontrol agent, *Streptomyces* sp. is also considered to have great potential to promote plant growth (Olanrewaju and Babalola 2019). It has been found that *Streptomyces* sp. has the ability to produce indole acetic-acid (IAA) phytohormone, solubilize phosphates and fix nitrogen (Gopalakrishnan *et al.* 2013). *Streptomyces* sp. PM9 has been studied for its ability to promote root number and root length in *Eucalyptus grandis* and *E. globules* by its high production of IAA (Salla *et al.* 2014). Inoculation of *Streptomyces* sp. to wheat (*Triticum aestivum*) increased plant growth by increasing phosphate solubilization ability and indole-3-acetic acid production. *Streptomyces* sp. also enhanced plant defense in wheat through the production of siderophore and chitinase to inhibit *Penicillium* sp. pathogen (Jog *et al.* 2014). Gopalakrishnan *et al.* (2013) also reported that inoculation of *Streptomyces* sp. significantly enhanced grain yield in sorghum by enhanced nitrogen source and availability of P in rhizosphere soil. In addition, Srivastava (2015) has shown that *Streptomyces* sp. has a capacity to protect chickpea (*Cicer arietinum* L.) plant from *Sclerotinia sclerotiorum* pathogen infection by increasing accumulation of antioxidant compounds such as phenolic compounds and antioxidant enzymes like catalase. Hence, this bacterial isolate was selected for further study especially in abiotic stress response.

Materials and Methods

Bacterial isolation

The studied bacterial strain was isolated by Dr. K.J. Duan from Tatung University. To perform strain identification, the bacterial strain was cultured in the solid medium of YCED (Casamino Acids Yeast Extract Glucose Agar) containing yeast extract 0.3 g, casamino acids 0.3 g, D-glucose 0.3 g, agar 20 g in 1,000 ml (de Vasconcellos *et al.* 2010). A single colony of the isolated bacterium was cultured in a liquid culture of the R2YE medium containing sucrose 103 g, K_2SO_4 0.25 g, $MgCl_2 \cdot 6H_2O$ 10.12 g, glucose 10 g, casamino acids 0.1 g, yeast extract 5 g in 1,000 ml (Shepherd *et al.* 2010). The bacterial strains were grown for 1 week at 28°C, and subsequently used for genomic DNA extraction.

Genomic DNA extraction

Genomic DNA extraction was prepared from a week old bacterial culture following the procedure of Wilson (2001). Genomic DNA fragments were obtained by

polymerase chain reaction (PCR) using primers fD1 and rD1. The fD1 forward sequence was AGAGTTGAT CCTGGCTCA, and rD1 reverse sequence was AAGAG GTGATCCAGCC. The PCR fragments were sequenced in both the sense and antisense directions using a 3730 DNA Analyzer (Applied Biosystems®; thermo-fisher.com). The obtained sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) program (Altschul *et al.* 1990). A phylogenetic tree was constructed using the 16 s rDNA sequences of isolated bacterial strains by the ClustalX method (Larkin *et al.* 2007).

Quantitative analysis of IAA production and phosphate solubility

The production of IAA from the bacterial isolate was tested with the colorimetric method described by Patten and Glick (2002). The bacterial isolate was inoculated in a test flask with 10 ml of R2YE culture medium containing one single colony of *Streptomyces* sp. with a supplement of $2 \text{ mg} \cdot \text{ml}^{-1}$ L-tryptophan at room temperature for a week. Bacterial culture was centrifuged for 15 min at 11,000 rpm. Two milliliters of the supernatant was mixed with 2 ml of Salkowski's reagent containing 36% (v/v) H_2SO_4 and $FeCl_3 \cdot 6H_2O$ 0.5 M, and incubated at room temperature for 30 min. The presence of IAA was determined by the development of a pink color and the IAA concentration was measured using a spectrophotometer at 530 nm and quantified according to an IAA standard curve. Phosphate solubilization bioassay was described by Tandon *et al.* (1968). Pikovskaya's (PVK) medium contained glucose $10 \text{ g} \cdot \text{l}^{-1}$, ammonium sulphate $0.5 \text{ g} \cdot \text{l}^{-1}$, sodium chloride $0.2 \text{ g} \cdot \text{l}^{-1}$, KCl $0.3 \text{ g} \cdot \text{l}^{-1}$, $FeSO_4 \cdot 7H_2O$ $0.03 \text{ g} \cdot \text{l}^{-1}$, $MnSO_4 \cdot 4H_2O$ $0.3 \text{ g} \cdot \text{l}^{-1}$, $Ca(PO_4)_2$ $5 \text{ g} \cdot \text{l}^{-1}$, yeast extract $0.4 \text{ g} \cdot \text{l}^{-1}$, and agar $15 \text{ g} \cdot \text{l}^{-1}$, with pH 7.0 was prepared. *Streptomyces* sp. was streaked on the middle surface of PVK medium and phosphate solubilizing activity was estimated after 1 to 2 weeks of incubation at room temperature for 60 min. The absorbance of the solution was determined at a wavelength of 420 nm. The phosphate concentration in the supernatant was calculated according to a standard curve created from known concentrations of KH_2PO_4 .

Analysis of the growth promotion effect of *Streptomyces* sp. on *Arabidopsis* seedlings

To investigate plants' responsiveness to growth-promoting effects of *Streptomyces* sp., 4-day-old *Arabidopsis thaliana* L. ecotype Columbia (Col-0) seedlings, grown in 1/2 Murashige and Skoog (MS) medium, were transferred to a new medium with one loop of bacterial inoculum and positioned approximately 2 cm below the root tips of seedlings. Petri dishes were placed vertically in a growth chamber at 23°C with a 16 h/8 h light/dark photoperiod. Ten-day-old

seedlings were analyzed for the growth-promoting effect of *Streptomyces* sp. Thirty plants were included in each treatment. The data are presented as the mean \pm SE from three independent experiments.

Analysis of the growth promotion effect of *Streptomyces* sp. and commercial biofertilizer in *Arabidopsis* and *Brassica* sp.

Two week-old *Arabidopsis* and 1-week-old *Brassica chinensis* L. seedlings in non-sterilized potting soil with fertilizer were prepared before treatment. An inoculant of 30 ml *Streptomyces* sp. with a density of 1×10^8 colony forming units CFU \cdot ml⁻¹ was utilized to treat *Arabidopsis* once a week for 3 consecutive weeks. A fertilizer (HYPONEX No. 2) with N-P-K ratio of 20-20-20 was foliar applied to both control and treated plants twice a week. Eight plants were included in each treatment. The data are presented as the mean \pm SE. from three independent experiments. Four-week-old *Arabidopsis* and *Brassica* sp. plants were analyzed for shoot growth and fresh weight.

Analysis of the growth promotion effect of *Streptomyces* sp. under abiotic stress

To analyze salt stress, 2-week-old *Arabidopsis* and 1-week-old *Brassica* sp. seedlings in non-sterilized potting soil were prepared before treatment. Salt stress was treated by 125 mM NaCl for *Arabidopsis*, and 150 mM NaCl for *Brassica* sp. For the heat treatment, 1-week-old *Arabidopsis* were treated at 40°C for 7 h for 4 days. Each day, after 7 h heat treatment, they were moved to a growth chamber with a long photoperiod (16 h light and 8 h dark) at 23°C. After 4 days of heat treatment, the plants continued to be grown in a growth chamber with a long photoperiod (16 h light and 8 h dark) at 23°C. For both abiotic stress treatments, an inoculant of 30 ml *Streptomyces* sp., with a bacterial population of $10^8 \cdot$ ml⁻¹ was applied twice a week to the plants. For the mock treatment, the same volume of water was applied to *Arabidopsis* seedlings. One day after the treatments, the water supply was withheld for 7 days until wilted seedlings appeared. At the end of the water withholding period, seedlings without wilted leaves were scored as survival plants. These water-restrained seedlings were re-watered, and the recovery fresh weight was measured 7 days after the water supply was renewed.

Determination of total chlorophyll content, hydrogen peroxide, lipid peroxidation level and antioxidant enzyme activity

To analyze chlorophyll content, leaf tissue of *Arabidopsis* mature plants (0.5 g) was homogenized in 20 ml chilled ETOH (95%) and stirred for 30 min.

The homogenate was centrifuged at 3,000 g for 5 min. The absorbance of the supernatant was recorded at 664 nm and 648 nm. Chlorophyll content (mg \cdot ml⁻¹) was estimated by the following formula $5.24 \times \text{OD}_{664} + 22.24 \times \text{OD}_{648}$. The procedures were described by Lichtenthaler (1987). To quantify H₂O₂ concentration, ferrous ion oxidation xylenol orange (FOX) solution was prepared following the procedures described by Delong *et al.* (2002). Fresh tissue (0.1 g) from mature soil-grown *Arabidopsis* was ground in 1 ml 80% ethanol, then centrifuged at 3,000 g for 10 min. One hundred microliters supernatant was added to FOX reagent and incubated at room temperature for 30 min in the dark. The absorbance of the solution at 560 nm was measured. The hydrogen peroxide (H₂O₂) concentration was calculated based on a standard curve generated from known concentrations of H₂O₂. To analyze the activity of catalase, 0.2 g of *Arabidopsis* leaf tissue was ground in liquid nitrogen and then added to 0.2 M potassium phosphate buffer (pH 7.8 with 0.1 mM EDTA). The supernatant of this extraction was used for the catalase analysis based on a previous publication (Aebi 1984) in which 0.1 ml of supernatant was added to 3 ml of analysis solution (0.1 mM EDTA, 0.1% H₂O₂, 100 mM potassium phosphate buffer pH 7.0). The catalase activity was expressed as micromole of H₂O₂ decomposed within 1 min with 1 g of fresh weight tissue. The lipid peroxidation level was determined in terms of malondialdehyde (MDA) content. Leaf tissue (0.1 g) from mature soil-grown *Arabidopsis* was ground in liquid nitrogen. One milliliter of 0.1% trichloroacetic acid (TCA) was added to precipitate protein. One milliliter of supernatant was taken for analysis. Two and a half milliliter of 0.5% TBA (thio-barbituric Q) in 20% TCA (3 g TCA + 0.075 g TBA + 15 ml ddH₂O) and the supernatant were added. The mixture was heated in a 95°C waterbath for 15 min and cooled immediately on ice to stop the reaction. The supernatant was taken and the absorbance at OD 532 nm and 600 nm was measured. Molandialdehyde concentration was estimated by subtracting the non-specific absorption at 600 nm from the absorption at 532 nm using an absorbance coefficient (155 mM \cdot cm⁻¹).

Protein extraction and western blot analysis

Total proteins extracted from mature plants were used as controls and those treated with *Streptomyces* sp. were prepared by homogenizing leaf tissues in ice cold buffer (50 mM Tris-Cl, 150 mM NaCl, 0.5% NP-40, 1 mM phenylmethanesulfonylfluoride (PMSF), 1 μ l protease inhibitor cocktail (Sigma-Aldrich). Fifteen micrograms of total protein were loaded onto 10% SDS-PAGE gels. Afterwards, the membrane was incubated with the polyclonal antibodies including glutamine synthetase (GS-1), cytosolic fructose-1.6-

-bisphosphatase (cFBPase). All antibodies were purchased from Agrisera (Vännäs, Sweden). The hybridization images were captured with Chemi-Smart 5000 (VilberLourmat, France).

Statistical analyses

The data were statistically analyzed with the Statistical Analysis System (SAS version 9.2) using an ANOVA and Tukey's test in which a p -value less than 0.05 indicated a significant difference. Each measurement was repeated at least three times independently.

Results

Strain identification and characterization of the isolated bacterium

The identification of bacterial strains was verified using the 16S rDNA sequence. A phylogenetic tree was constructed using the ClustalX method (Larkin *et al.* 2007) which confirmed that the bacterial strain was *Streptomyces* sp. (Fig. 1A). *Streptomyces* sp. is known to have great potential for promoting plant growth and as a biocontrol agent (Evangelista and Martínez 2013; Olanrewaju and Babalola 2019). Hence, this bacterial isolate was selected for further study especially for its abiotic stress response. In this study, we found that *Streptomyces* sp. induced catalase antioxidant enzyme production and was able to secrete large

amounts of IAA $83.05 \mu\text{g} \cdot \text{ml}^{-1}$ as well as solubilize phosphate $12.64 \mu\text{g} \cdot \text{ml}^{-1}$ (Fig. 1B). At the molecular level we found that *Streptomyces* sp. changed the expression of GS1, cFBPase, and Lhcb1 genes which contributed to increased plant growth in *Arabidopsis* and *Brassica* sp.

Analysis of the growth promotion effect of *Streptomyces* sp. on *Arabidopsis* seedlings

The bacterial isolates were co-cultured with the 4-day-old seedlings that had been grown in 1/2 Murashige and Skoog (MS) medium and transferred to a new medium with one loop bacterial inoculum positioned approximately 2 cm below the root tips of seedlings for 10-day-old seedlings. The *Streptomyces* sp. inoculation altered root architecture of *Arabidopsis* seedlings by significantly increased lateral root numbers of *Arabidopsis*. Moreover, the 3-week-old soil-grown *Arabidopsis* treated with *Streptomyces* sp. exhibited increases in fresh weight and chlorophyll contents (Figs. 2A and B).

Growth promotion effect of *Streptomyces* sp. and commercial biofertilizer on *Arabidopsis* and *Brassica* sp.

This study found that *Streptomyces* sp. stimulated the development of lateral roots in *Arabidopsis* (Fig. 2A), which in turn caused an increase in the fresh weight in *Arabidopsis* and *Brassica* sp. (Figs. 3 and 5). To test whether isolated bacterial strains and biofertilizer can

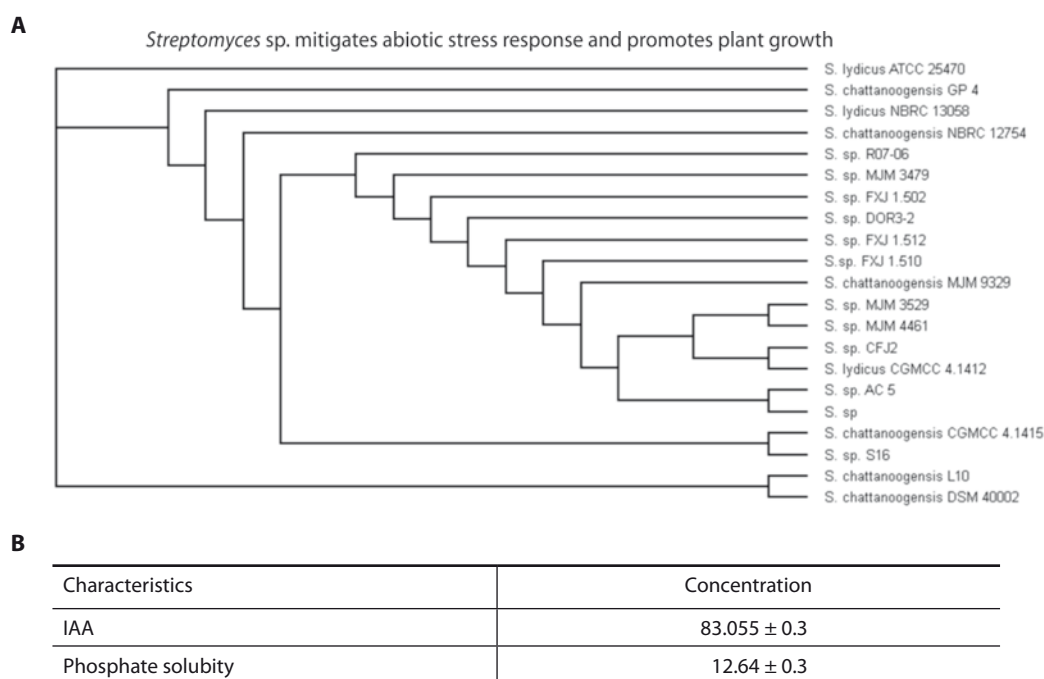


Fig. 1. Characterization of *Streptomyces* sp. (A) The 16S rRNA sequences from various *Streptomyces* strains were used for construction of a phylogenetic tree using the ClustalX method. (B) Quantification of indole-acetic acid (IAA) and solubilizing phosphate produced by the activities of *Streptomyces* sp.

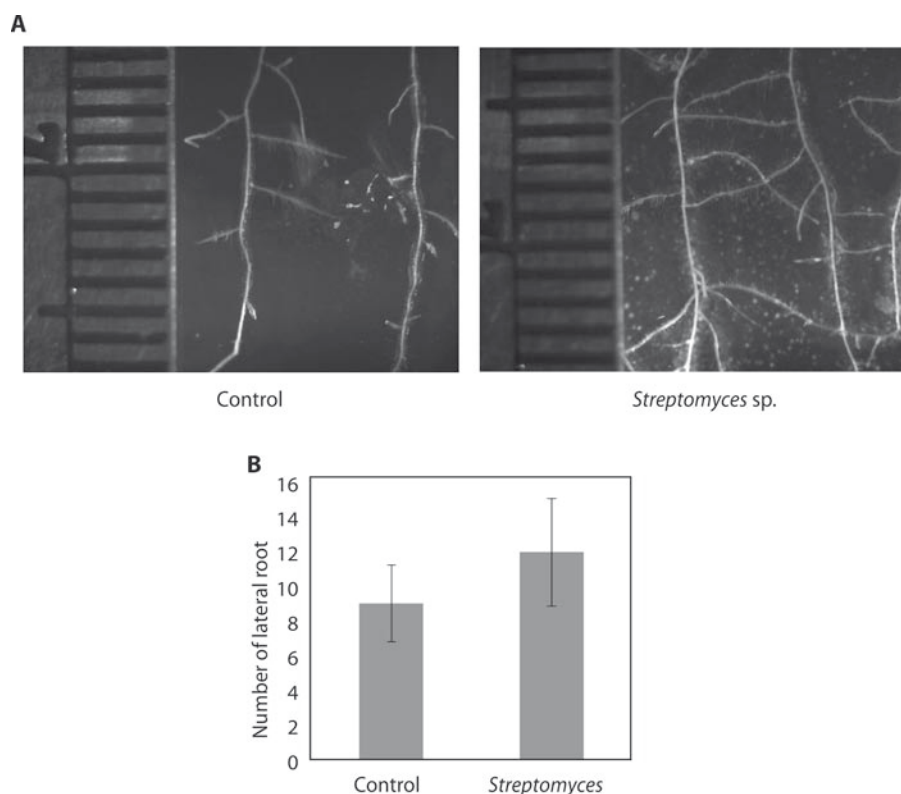


Fig. 2. *Streptomyces* sp. affected plant growth. Four-day-old *Arabidopsis* seedlings grown in 1/2 Murashige and Skoog (MS) medium and transferred to a new medium with one loop bacterial inoculum positioned approximately 2 cm below root tips of seedlings and grown for an additional 6 days. The 10-day-old seedlings exhibited changes in root architecture (A) and increased lateral root hair number (B)

promote plant growth, *Arabidopsis* was treated with *Streptomyces* sp. and HYPONEX No. 2 biofertilizer. Fresh weight and chlorophyll content were investigated and showed significant increases compared to the control (Figs. 3B and C). At a molecular level *Streptomyces* sp. induced protein accumulation including nitrogen assimilation such as GS1, carbohydrate metabolism such as cFBPase, and the light-harvesting chlorophyll such as Lhcb1 protein (Fig. 4). Besides treating *Arabidopsis*, the application of *Streptomyces* sp. was tested in *Brassica* sp. as well. In the field there was increased vegetative growth of this vegetable after 3 weeks of treatment (Fig. 5A). *Streptomyces* sp. increased *Brassica* sp. fresh weight compared to the control (Fig. 5B). Furthermore, *Streptomyces* sp. and biofertilizer treatment can increase plant production compared to fertilizer only. Isolated bacterial strains and biofertilizer produced the highest fresh weight of *Brassica* sp.

***Streptomyces* sp. increased tolerance to salt stress and heat stress**

Under conditions of abiotic stress, salt stress treatment (125 mM NaCl) in *Arabidopsis* led to a high production of reactive oxygen species, H_2O_2 and MDA as a lipid peroxidation. Hydrogen peroxide and lipid peroxidation concentration under salt treatment were significantly

higher than those under other treatments (Fig. 6). However, as shown in Figures 6C and D hydrogen peroxide and lipid peroxidation concentration were significantly reduced as a result of inoculation of *Streptomyces* sp. Catalase enzyme may act as a scavenger of ROS for mitigating the injury in plants (Fig. 6E). *Streptomyces* sp. promoted salt stress tolerance in *Brassica* sp. as well. The effect of salt stress on Chinese cabbage has been observed. Salt stress treatment with 150 mM NaCl produced small shoot growth in *Brassica* sp. (Fig. 7). However, treatment with bacteria *Streptomyces* sp. improved the survival of this plant, demonstrating a protective effect of these bacteria. Recovery to a healthier phenotype and continued growth was poor in untreated plants while bacteria treated plants showed a good recovery after salt treatment. The results showed that inoculation of *Streptomyces* sp. had higher fresh weight than the control treatment, as seen by greater growth of shoots (Figs. 7A and B). The identification of a *Streptomyces* sp. effect on improving heat stress tolerance can be seen in Figure 8A. Under heat stress conditions, *Arabidopsis* shoot growth was smaller and more yellow after exposure to a temperature of 40°C for 7 h for 4 days. The ability of *Streptomyces* sp. to enhance plant tolerance toward heat stress was investigated. Under heat stress conditions, fresh weight and chlorophyll content of *Arabidopsis* was measured.

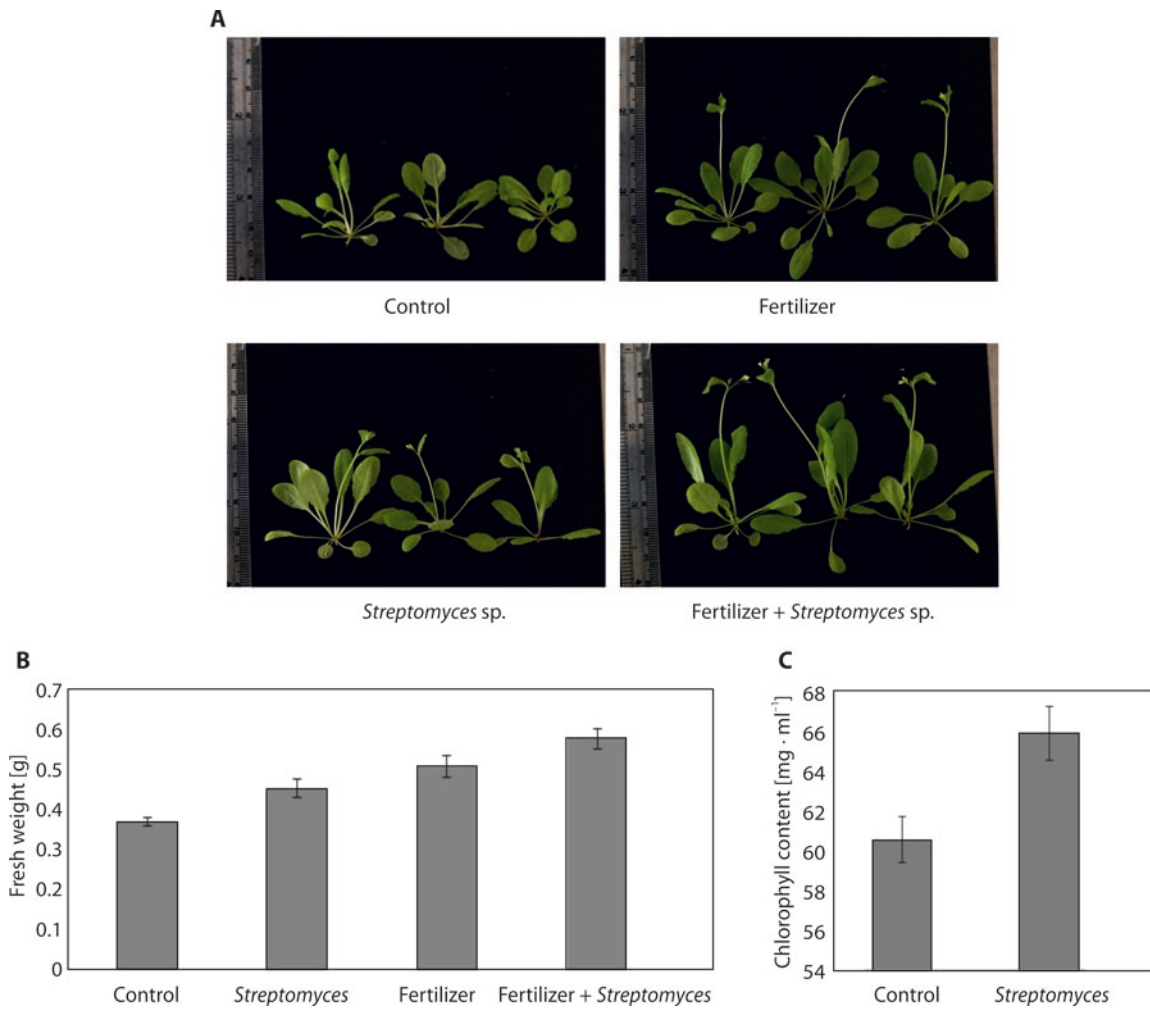


Fig. 3. *Streptomyces* sp. affected *Arabidopsis* plant growth. Two- and 1-week-old *Arabidopsis* and *Brassica* sp. seedlings grown in pots, respectively, were inoculated with 1×10^8 colony forming units $\text{CFU} \cdot \text{ml}^{-1}$ *Streptomyces* sp. once a week for 3 consecutive weeks. A fertilizer (HYPONEX No 2) with N-P-K ratio of 20-20-20 was foliar applied to both control and treated plants twice a week. Four-week-old *Arabidopsis* plants were analyzed for shoot growth and fresh weight. (A) *Streptomyces* sp. promoted vegetative growth in *Arabidopsis*; (B and C) *Streptomyces* sp. increased fresh weight and chlorophyll content in *Arabidopsis*

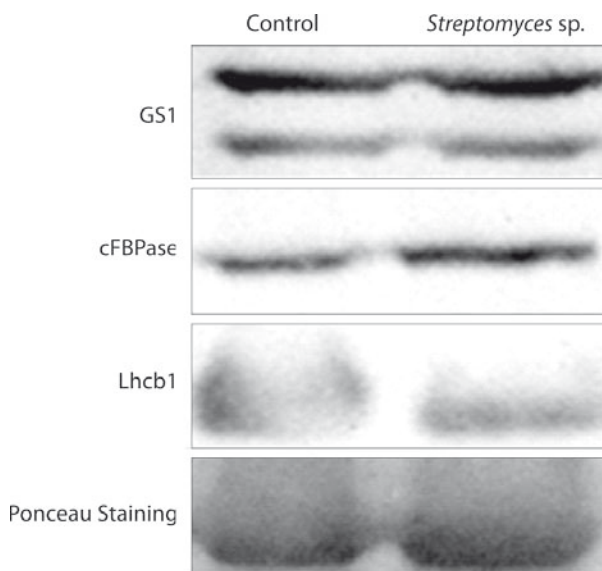


Fig. 4. *Streptomyces* sp. induced protein accumulation in *Arabidopsis*. Total proteins isolated from *Arabidopsis* seedlings inoculated with bacteria inoculants were hybridized with antibodies derived from glutamine synthetase 1 (GS1), cytosolic fructose-1,6-bisphosphatase (cFBPase), and light-harvesting chlorophyll a/b binding (Lhcb1) protein accumulation in *Arabidopsis*. Ponceau staining of transferred proteins was used to check equal loading of protein samples

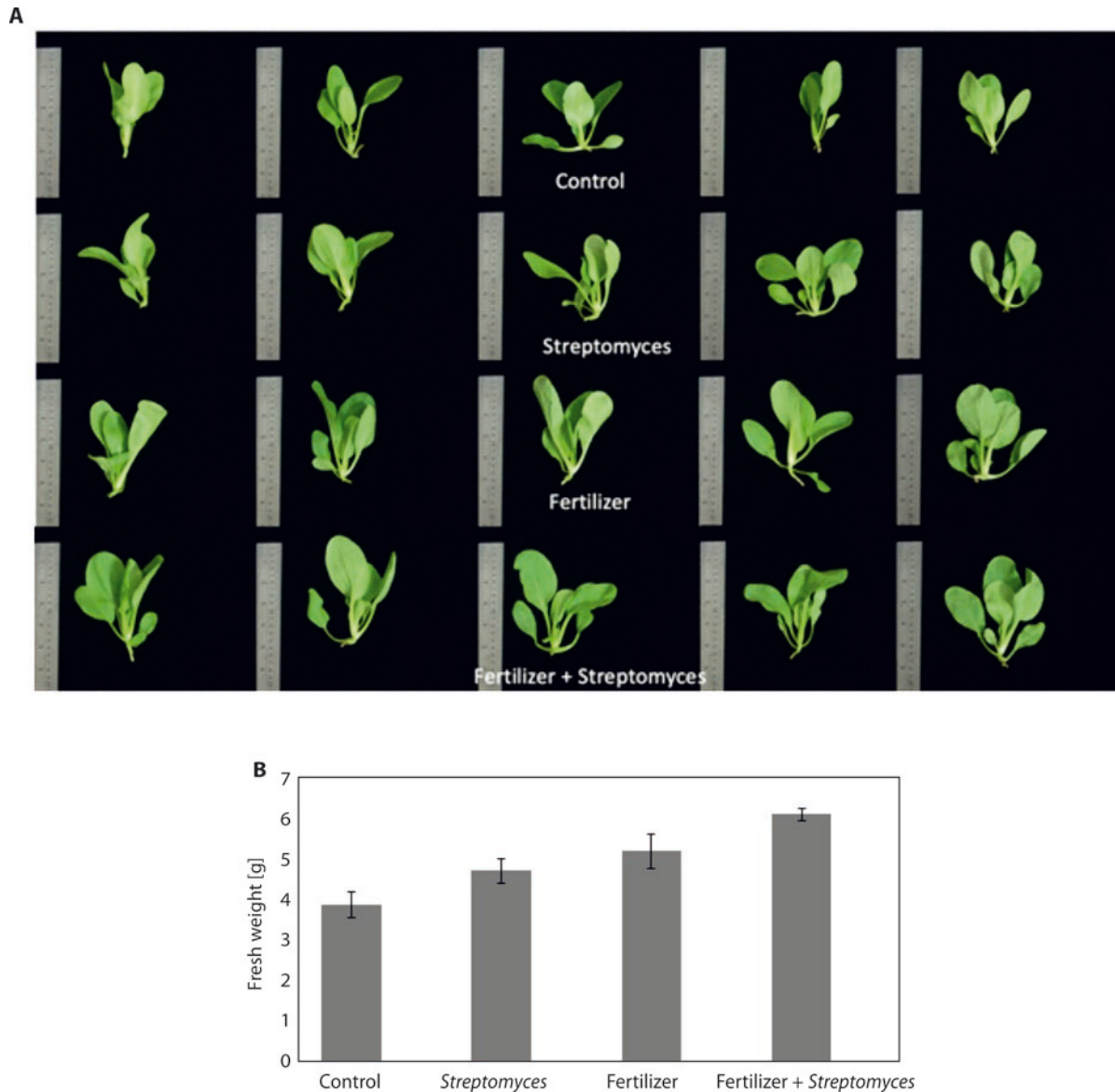


Fig. 5. *Streptomyces* sp. affected *Brassica* sp. plant growth. One-week-old *Brassica* sp. seedlings grown in pots were inoculated with 1×10^8 colony forming units $\text{CFU} \cdot \text{ml}^{-1}$ *Streptomyces* sp. once a week for 3 consecutive weeks. A fertilizer (HYPONEX No 2) with a N–P–K ratio of 20–20–20 was foliar applied to both control and treated plants twice a week. Four-week-old *Brassica* sp. plants were analyzed for shoot growth and fresh weight. (A) *Streptomyces* sp. promoted vegetative growth in *Brassica* sp. (B) *Streptomyces* sp. increased fresh weight in *Brassica* sp.

Inoculation of *Streptomyces* sp. increased fresh weight (Fig. 8B) and chlorophyll (Fig. 8C) content in *Arabidopsis*.

Discussion

Abiotic stress, in this scenario, salt and heat stress caused various physiological and biological changes in basil plants, one of which was the accumulation of reactive oxygen species in the cell. Reactive oxygen radicals are toxic and may result in a series of injuries to plant metabolism that affect the growth and

productivity of the plant. The results of this study showed that, salt and heat stress increased hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and decreased the fresh weight and chlorophyll content. However, inoculation with *Streptomyces* sp. could efficiently be used to improve growth, antioxidant status and photosynthetic pigments under salt and heat stress. Under salt stress, *Streptomyces* sp. significantly improved catalase enzyme activity in the leaves, and increased fresh weight and plant vegetative growth. Under heat stress the chlorophyll content in leaves was increased by inoculation with *Streptomyces* sp.

Under salt stress treatment (125 mM NaCl) in *Arabidopsis*, the production of H_2O_2 and MDA were

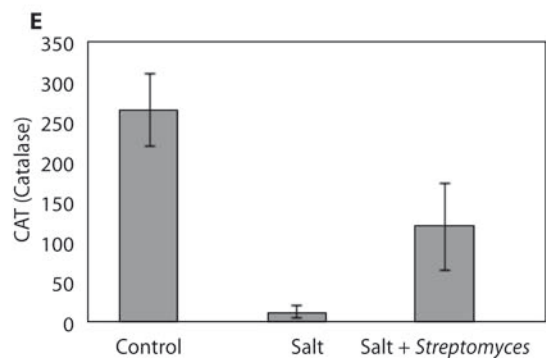
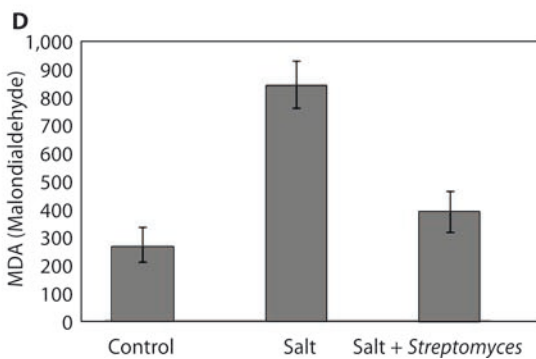
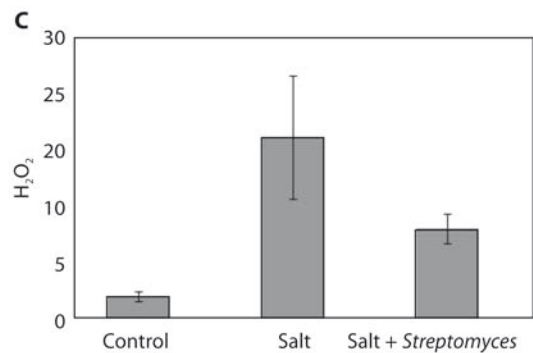
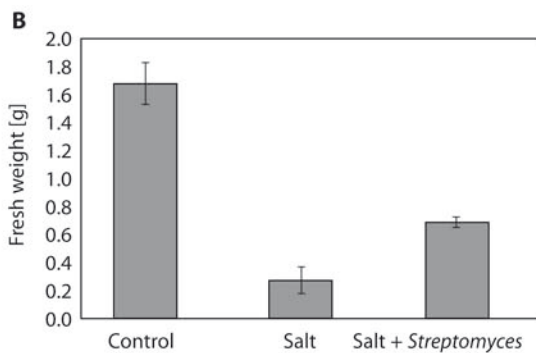
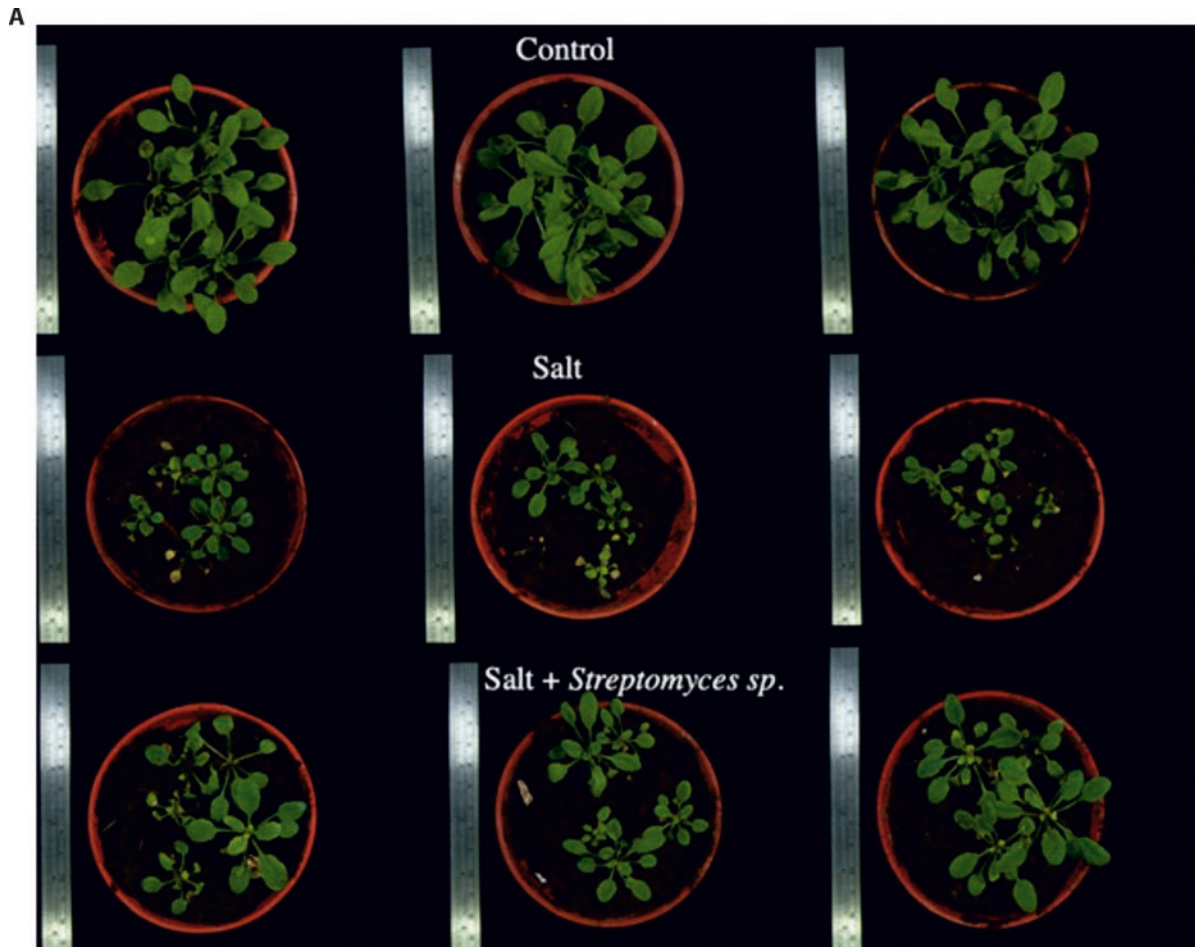


Fig. 6. *Streptomyces* sp. promoted salt stress tolerance in *Arabidopsis*. Two-week-old seedlings grown in pots with 125 mM NaCl, inoculated with *Streptomyces* sp. (1×10^8 CFU · ml⁻¹) for 3 consecutive weeks, exhibited increments in several physiological systems including fresh weight (B), hydrogen peroxide (C), malondialdehyde (D) and catalase antioxidant enzymes (E)

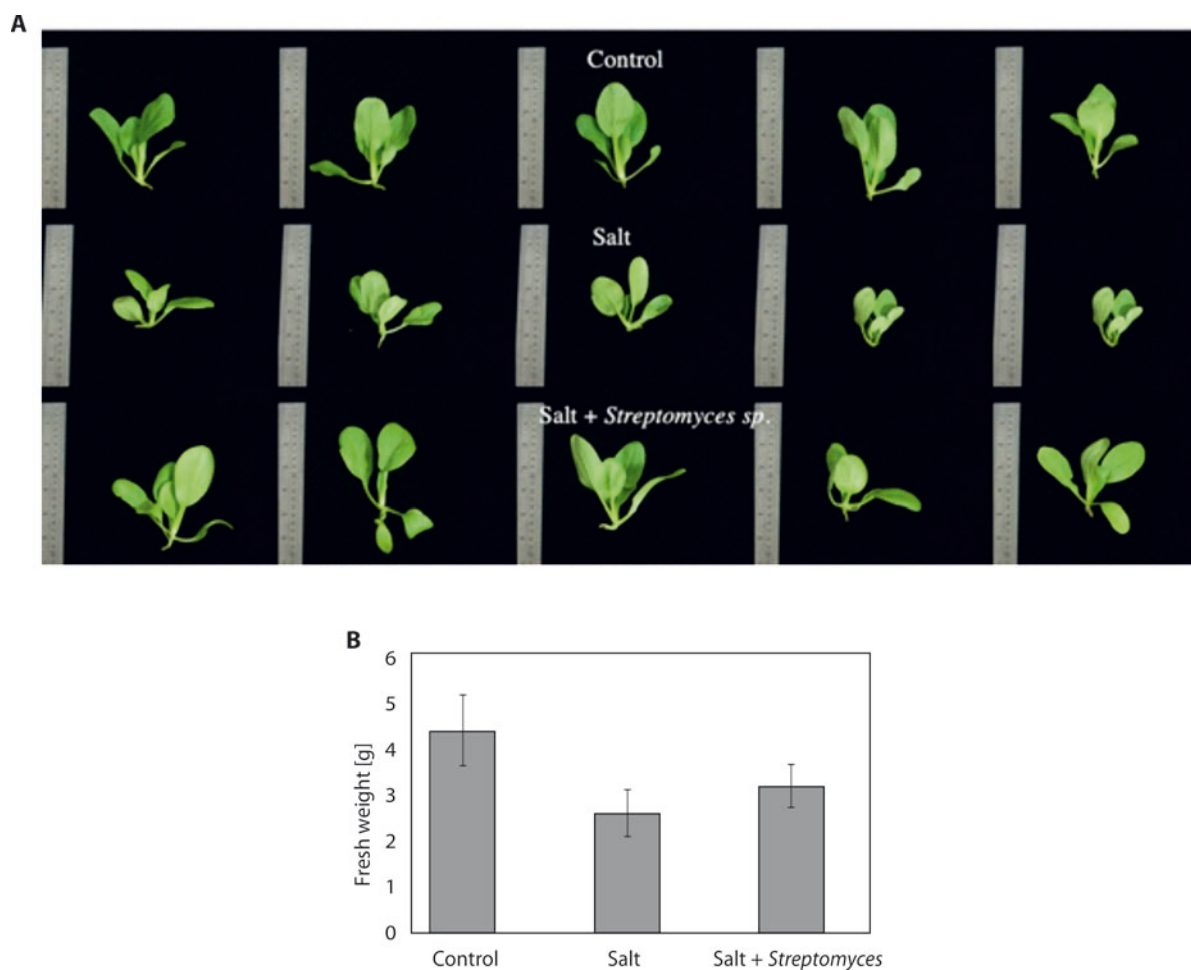


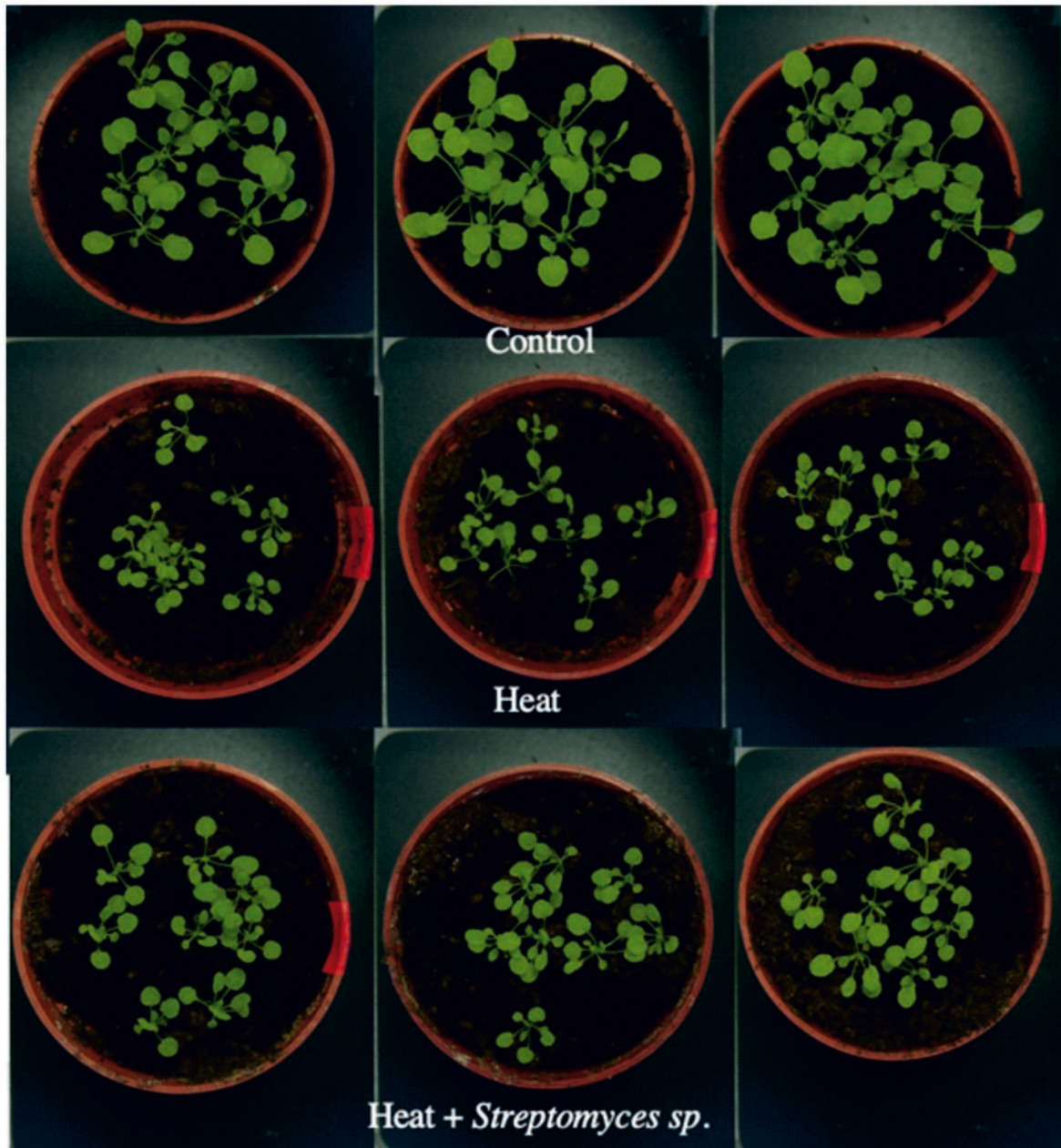
Fig. 7. *Streptomyces* sp. promoted salt stress tolerance in *Brassica* sp. (A) *Streptomyces* sp. promoted vegetative growth in *Brassica* sp. under salt stress. (B) One-week-old *Brassica* sp. seedlings grown in pots with 150 mM NaCl, inoculated with *Streptomyces* sp. ($1 \times 10^8 \cdot \text{ml}^{-1}$) for 3 consecutive weeks, exhibited increments in fresh weight

significantly higher than under other treatments. However, these were significantly reduced as a result of inoculation with *Streptomyces* sp. (Figs. 6C and D). This study showed that *Streptomyces* sp. induced catalase enzyme production in *Arabidopsis* under salt stress treatment (Fig. 4E). This result suggests that catalase antioxidant enzyme activity by *Streptomyces* sp. isolates is able to confer abiotic stress tolerance and increase plant growth in *Arabidopsis* (Fig. 6) and *Brassica* sp. (Fig. 7). Catalase enzyme may act as a scavenger of ROS for mitigating the injury in plants (Fig. 6E). Catalase antioxidant enzyme prevented the formation of H_2O_2 under abiotic stress in plants (Sharma and Ahmad 2014). There are many reports about the relationship between enhanced antioxidant enzyme activities and increased resistance to environmental stresses in several plant species, such as rice (Rossatto *et al.* 2017), vegetable crops (Kusvuran *et al.* 2016), sugar beet (Karagöz *et al.* 2018), wheat (Mutlu *et al.* 2018) and barley (Pakar *et al.* 2016).

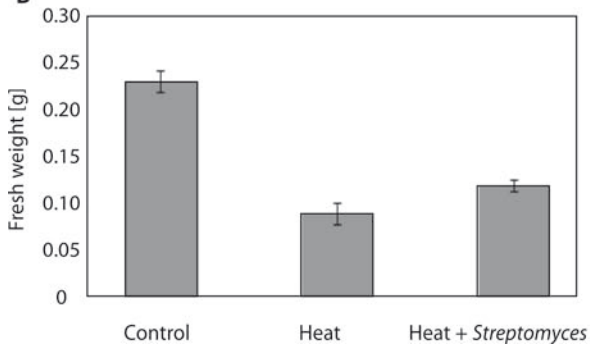
In addition to its antioxidant production, PGPR's ability to increase plant growth is due to its ability to

secrete very functional hormones for plant growth. In this study, we found that *Streptomyces* sp. is able to secrete high amounts of IAA $83.05 \mu\text{g} \cdot \text{ml}^{-1}$ (Fig. 2) and can solubilize phosphate $12.64 \mu\text{g} \cdot \text{ml}^{-1}$ (Fig. 3). The ability of this isolate to increase lateral root number in *Arabidopsis* is closely related to the IAA and phosphate solubilisation production, which was produced by *Streptomyces* sp. isolates (Fig. 2). *Streptomyces* sp. isolates that significantly promoted physiological responses such as fresh weight and chlorophyll content in *Arabidopsis* and *Brassica* sp. were able to solubilize phosphate (Fig. 3). Varying studies have found that IAA in PGPR can induce root growth in plants (Casán *et al.* 2014; Poupin *et al.* 2016) and the production of phosphate solubilisation of inoculants simultaneously increases P uptake by the plant and crop yield (Kalayu 2019). The principal mechanism for mineral phosphate solubilization is the production of organic acids that are used in the mineralization of organic phosphorus in soil. This ability provides a promising alternative of phosphate biofertilizers in fields to reduce the use of phosphate chemical fertilizers.

A



B



C

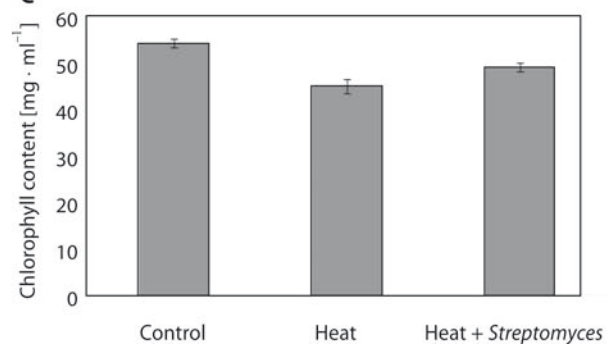


Fig. 8. *Streptomyces* sp. promoted heat stress tolerance in *Arabidopsis*. One-week-old *Arabidopsis* were treated at 40°C for 7 h for 4 days. After 4 days of heat treatment, plants continued to be grown in a growth chamber with a long photoperiod (16 h light and 8 h dark) at 23°C. An inoculant of 30 ml *Streptomyces* sp. with a bacterial population of 10⁸ · ml⁻¹ was applied twice a week to plants. One day after the treatments, the water supply was withheld for 7 days until wilted seedlings appeared. At the end of the water-withholding period, seedlings without wilted leaves were scored as survival plants. These water-restrained seedlings were re-watered, and their recovery fresh weight was measured 7 days after being rewatered. (A) *Streptomyces* sp. promoted vegetative growth in *Arabidopsis* under heat stress. (B and C) *Streptomyces* sp. increased fresh weight and chlorophyll content in *Arabidopsis* under heat stress

Moreover, at a molecular level *Streptomyces* sp. induced protein accumulation including nitrogen assimilation, such as GS1, carbohydrate metabolism, such as cFBPase, and the light-harvesting chlorophyll, such as Lhcb1 protein (Fig. 5). In this study we found changes in the expression of GS1, cFBPase, and Lhcb1 genes (Fig. 5). Glutamine synthetase (GS1) is a key enzyme in nitrogen assimilation and metabolism in higher plants. There is no doubt that nitrogen assimilation is an important process in the formation of organic nitrogen compounds from inorganic nitrogen compounds present in the environment. Nitrogen is a major component of chlorophyll, the compound by which plants use sunlight energy to produce sugars from water and carbon dioxide in photosynthesis. It is also a major component of amino acids, the building blocks of proteins. Cytosolic fructose-1, 6-bisphosphatase (cFBPase) is a key component in photosynthetic sucrose biosynthesis. Photoassimilated carbons are converted to sucrose in green plant leaves and distributed to provide carbon and energy. cFBPase overexpression lines exhibited enhanced growth with larger rosette sizes and increased fresh weights than with wild-type (WT) plants (Cho *et al.* 2012). Light-harvesting chlorophyll such as Lhcb1 protein is one of the most abundant proteins of the chloroplast in plants. It roughly accounts for half of the chlorophyll involved in photosynthesis. The main function of Lhcb1 is collecting and transferring light energy to photosynthetic reaction centers. The enhancement of GS1, cFBPase, and Lhcb1 gene protein expression levels by *Streptomyces* sp. treated plants contributed to increased plant growth in *Arabidopsis* and *Brassica* sp.

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