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RESEARCH ARTICLE

Plant growth promoting bacteria *Bacillus subtilis* promote growth and physiological parameters of *Zingiber officinale* Roscoe

D. Jabborova[1,2*], Y. Enakiev[3], K. Sulaymanov[1,4], D. Kadirova[5], A. Ali[6] & K. Annapurna[2]

[1]Institute of Genetics and PEB, Academy of Sciences of Uzbekistan
[2]Division of Microbiology, ICAR-Indian Agricultural Research Institute, India
[3]Agricultural Academy, "Nikola Pushkarov" Institute of Soil Science, Agrotechnology and Plant Protection, Sofia 1331, Bulgaria
[4]Tashkent State Agrarian University, Uzbekistan
[5]Termez State University, Termez, Uzbekistan
[6]Department of Life Sciences, University of Mumbai, Vidyanagari, Santacruz (East), Mumbai 400 098, India
**Email: dilfuzajabborova@yahoo.com*

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ABSTRACT

Ginger (*Zingiber officinale* Roscoe) is an important medicinal crop grown for its aromatic rhizome which is used as a spice, food, flavouring agent and medicine. It has been characterised for its hypoglycemic, hypotensive, antioxidant and antibiotic properties. This study was conducted to determine the impact of plant growth-promoting potential of bacterial strain *Bacillus subtilis* L2 on plant growth and physiological properties of ginger. The experiment was carried out in randomised block design with three replications in pot experiments. The plants were grown in greenhouse conditions for three months. The results showed that at 8 and 12 weeks after planting (WAP) bacterial inoculation increased plant height, leaf length, number of leaves per plant and leaf width. Inoculation with *B. subtilis* L2 significantly increased plant height by 16, 20 and 18% compared to control at 4, 8 and 12 WAP. At 8 and 12 WAP, leaf length significantly raised by *B. subtilis* L2 as compared to uninoculated control. *B. subtilis* L2 significantly increased the number of leaves per plant and leaf width by 30 and 21% respectively when comparing with non-inoculated plants at 8 WAP. The percentage increase in chlorophyll content resulted from the inoculation with *B. subtilis* L2 over the control was 10.5%, 15.5% and 18.4% at 4, 8 and 12 WAP respectively. It is concluded that there is a significant positive effect of inoculation with *B. subtilis* L2 on the growth of ginger. *B. subtilis* L2 strain can be used as a potential agent or bio-fertiliser for stimulation of ginger growth.

Introduction

Ginger (*Zingiber officinale* Roscoe) is a spice and medicinal plant belonging to the family Zingiberaceae family. Ginger has been utilised in folk medicine in India and China. Especially in India, the fresh and dry root of ginger is widely used in medicine, food industry and also as a vegetable. It is used to make gingerbread, sweets, cakes and snacks in western countries. It has been long time utilised in folk medicine as a drug considering colds, sore throats, coughs, asthma and arthritis (1–5).

Ginger is rich in beneficial minerals such as phosphorus, potassium and calcium which play an important role in human physiological processes. These substances play an essential role in boosting human immunity and maintaining good health. Ginger root contains biologically active compounds. It also contains carbohydrates (6), fats (7), proteins, vitamins (8) and minerals (9), organic acids (10), amino acids (11), monoterpenoids (camphene, cineole, borneol, citral, curcumin and linalol) and gingerol (12–14).

Crop cultivation and disease control can be achieved through the use of chemical fertilisers and pesticides; their long-term use has a damaging effect on plant ecology (15). Consequently, plant growth-promoting bacteria (PGPR) have been used to improve soil fertility, plant development and plant health (16–18). PGPR play a leading part in improving plant

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growth through a variety of mechanisms. They can contribute to plant growth includes nitrogen uptake, synthesis of phytohormones, minerals solubilisation, siderophore production, inhibition of plant ethylene synthesis, production of antibiotics and the production of enzymes (19–26). These rhizobacteria have been found to improve plant growth (27–35). Notably, representatives of the *B. subtilis* are recognised for their helpful action on the growth of plants (36). The inoculation with *Bacillus* sp. increased the plant tolerant to stress and produces various plant hormones for development enhancement (37). The ability of *Bacillus subtilis* PTS-394 strain increased tomato (*Solanum lycopersicum*) height and root growth (38). We conducted the present study to determine the impact of plant growth-promoting *Bacillus subtilis* on growth, transpiration rate and chlorophyll content of ginger.

Materials and Methods

Bacterial strain

Bacillus subtilis L2 strain were obtained from the Laboratory of Medicinal Plants Genetics and Biotechnology, Institute of Genetics and Plant Experimental Biology. *B. subtilis* L2 was cultured using nutrient agar containing beef extract 3 gm, peptone 5 gm, agar 15 gm, water 1000 ml. Nutrient broth containing beef extract 3 gm, peptone 5 gm, water 1000 ml was used for the rhizome inoculations.

Phosphate solubilisation

Phosphate solubilisation by bacteria phosphate solubilising ability was detected by spot inoculating pure isolated bacterial strain on the Pikovskaya medium contained l^{-1} : glucose, 10 gm; $Ca_3(PO_4)_2$, 5 gm; $(NH_4)_2SO_4$, 0.5 gm; NaCl, 0.2 gm; $MgSO4.7H_2O$, 0.1 gm; KCl, 0.2 gm; beef extract, 0.5 gm; $MnSO_4$. H_2O, 0.002 gm; and $FeSO_4.7H_2O$, 0.002 gm (39) and incubated at 28 °C for three to seven days along with the control plates. The uninoculated plates served as control. All the inoculations were done in triplicate. Phosphate solubilisation by bacterial strain was tested by their ability to solubilise inorganic phosphate. After seven days the formation of clearing zones was evaluated.

Indole acetic acid (IAA) production

The strain was grown for 48 hr on their respective media at 28. The fully grown strain was centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). The development of a pink colour indicated IAA production (40).

Siderophore production

The strain was spotted on the Chrome azurol S agar media components are as follows: 100 mM Pipes, 18 mM NH_4Cl, 22 mM KH_2PO_4, 2% (wt/vol) NaCl, 0.3% casamino acids, 0.2% (wt/vol) glucose, 10 μM $FeCl_3$, 58 μM CAS and 80 μM HDTMA (41). The strain was incubated at 28 °C for 5–7 days (41). The development of yellow-orange hallow zone around the bacterial spot has been considered as a positive indication for siderophore production.

Greenhouse experiment

Ginger rhizomes were used for plant growth pot experiments. *Bacillus subtilis* L2 strain were used for inoculation of the sterilised rhizome. One ml of each culture was pelleted by centrifugation, and cell pellets washed with 1 ml phosphate-buffered saline (PBS; 20 mM sodium phosphate, 150 mM NaCl, pH 7.4) and resuspended into PBS. The suspensions used for the inoculation was adjusted to the final concentration of approximately 10^7 cells/ml. Rhizomes were cultivated into plastic pots of soil. Soil samples were collected from the field of the Institute of Genetics and Plant Experimental Biology. For planting, small shallow pits were made and the rhizomes of 20 gm each of ginger were placed in the pits. The rhizomes were placed 4 cm deep in the pits and the soil pressed over it. Plants were grown for 12 weeks in greenhouse conditions. After four, eight and twelve weeks, plant height, the number of leaves per plant, the length of leaf, the width of leaf, chlorophyll content and transpiration rate were determined. The fresh tissues from leaf were ground in a mortar and pestle containing 80% acetone for chlorophyll content. The optical density (O.D.) of the solution was read at 663–645 nm using a spectrophotometer. The chlorophyll content present in the extract (mg g^{-1} tissue) was calculated by using the following calculation (42):

Total chlorophyll content =20.2xO.D.645+18.2 O.D.633

Statistical analysis

Experimental data were analysed with the StatView Software using ANOVA. The significance of the effect of treatment was determined by the magnitude of the F value ($P < 0.05$).

Results and Discussion

The results illustrated in Table 1 shows plant growth-promoting characteristics of *Bacillus subtilis* L2 strain. Further *B. subtilis* produced siderophore, IAA and caused P-solubilization. (Table 1). In the present investigation, *B. subtilis* L2 has shown positive results for plant growth-promoting characteristics such as IAA, phosphate solubilisation and siderophore production.

Table 1. Plant growth-promoting characteristics of *B. subtilis* L2 strain

Strain	IAA	Phosphate solubilisation	Siderophore production
B. subtilis L2	+	+	+

+ Positive for the test;- Negative for the test

The results also showed that at 8 and 12 weeks after planting (WAP), the bacterial inoculation increased the height of the plant. The maximum plant height was observed at 12 WAP (Fig. 1a). Plant height increased notably by 53% at 8 WAP compared to 4 WAP. Ginger plant height peaked at 12 weeks compared to 4 and 8 WAP of inoculation. The height of the plant significantly increased by 37% and 112% compared to control at 4 and 8 WAP. Interestingly, bacterial inoculation increased plant height at 4, 8

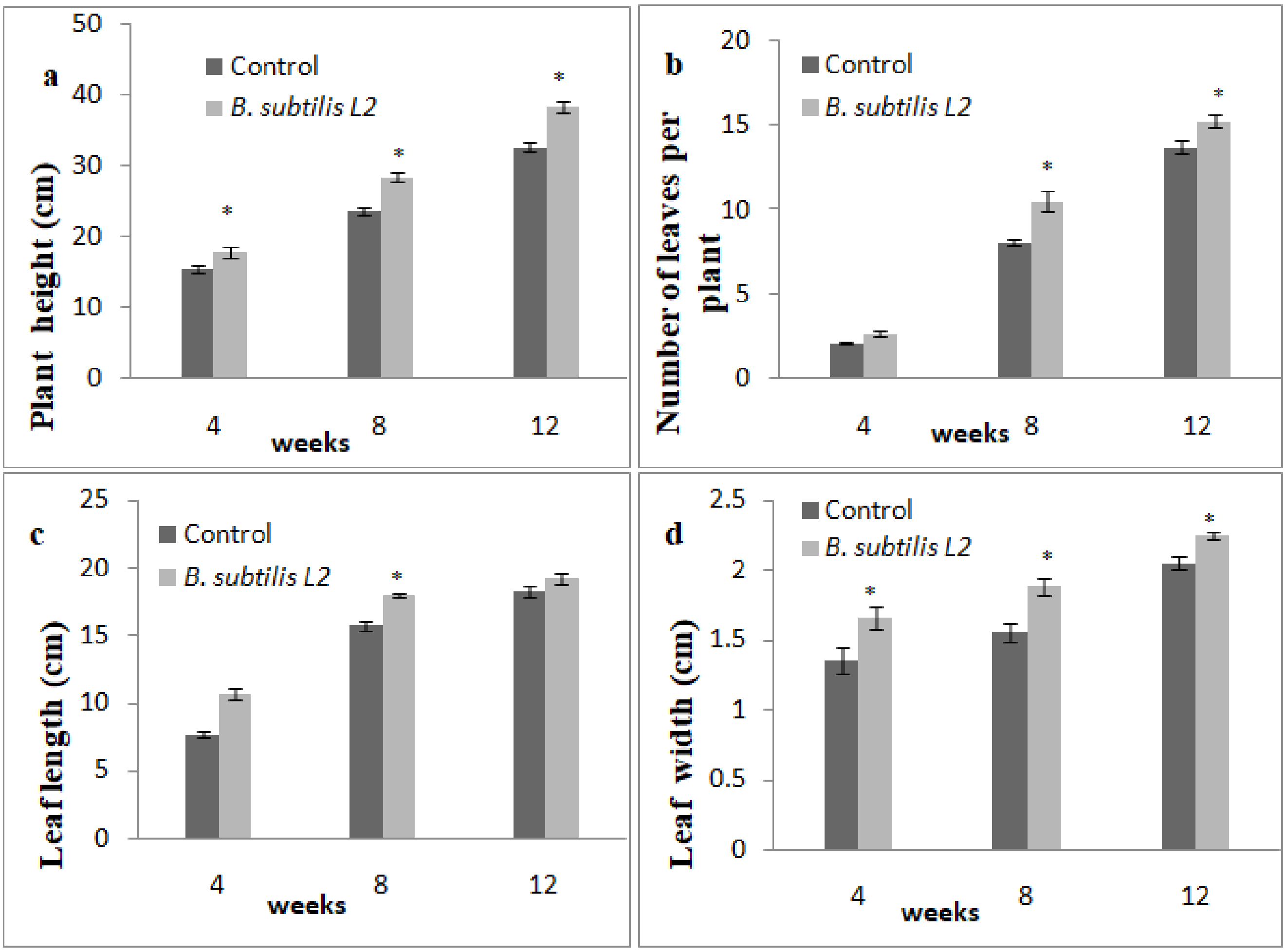

Fig. 1. Effect of inoculation with *Bacillus subtilis* L2 on plant height (a), number of leaves per plant (b), leaf length (c) and leaf width (d) of Ginger (*Zingiber officinale*).

and 12 WAP. Inoculation with *B. subtilis* L2 significantly increased plant height by 16, 20 and 17% compared to control at 4, 8 and 12 WAP.

The number of leaves per plant at 12 WAP is best and significantly different with 4 WAP and 8 WAP (Fig. 1b). At 12 WAP, the number of leaves significantly raised by 69% than uninoculated control. Comparatively a smaller number of leaves at four weeks confirmed that the total leaf number peaked at eighth and twelfth weeks. *B. subtilis* L2 significantly increased the number of leaves per plant by 29 and 10% respectively when comparing with non-inoculated plants at 8 and 12 WAP.

The length of leaves per plant at 12 WAP is best and significantly different with 4 and 8 WAP (Fig. 1c). The leaf length significantly increased by 139% and 16% compared to control at 4 and 8 WAP. Bacterial inoculation improved leaf length at 4, 8 and 12 WAP. Inoculation with *B. subtilis* L2 significantly enhanced leaf length by 39 and 14% comparing with uninoculated plants at 4 and 8 WAP.

The leaf width sharply increased during 4, 8 and 12 weeks in both uninoculated and inoculated conditions. The leaf width peaked at 12 WAP compared to uninoculated control 4 and 8 WAP (Fig. 1d). Inoculation with *B. subtilis* L2 significantly increased the leaf width at 4, 8 and 12 WAP. *B. subtilis* L2 enhanced the leaf width significantly by 22%, 215 and 9% compared to control uninoculated at 4, 8 and 12 WAP.

Regarding the effect of *B. subtilis* L2, it was noted that *B. subtilis* L2 treatment increased the transpiration rate significantly over the control at 4, 8 and 12 WAP. The transpiration rate gradually increased during 4 WAP control treatment. *B. subtilis* L2 treatment showed an increase of 8.6%, 9.8% and 10.0% over the control at 4, 8 and 12 WAP respectively (Fig. 2a).

The inoculation of *Bacillus subtilis* L2 improved the chlorophyll content compared to the control at 8 and 12 WAP (Fig. 2b). Inoculation with *B. subtilis* L2 also increased slightly the chlorophyll content at 4 WAP. The percentage increase in chlorophyll content resulted from the inoculated with *B. subtilis* L2 over the control was 10.5%, 15.5% and 18.4% at 4, 8 and 12 WAP respectively.

Discussion

Plant growth-promoting bacteria (PGPR) established to mass-produce a wide diversity of plant-helpful metabolites such as IAA, siderophore and P solubilisation that support in plant growth and the portly energy of the plant (28–31). Mass-production of IAA (Indole-3-acetic acid), siderophore and P solubilisation have been informed in numerous species of *Bacillus*, *Pseudomonas* and *Bradyrhizobium* including *B. japonicum* (24), *B. subtilis* (18) and *P. putida* (38, 43–45). In the present investigation, *B.*

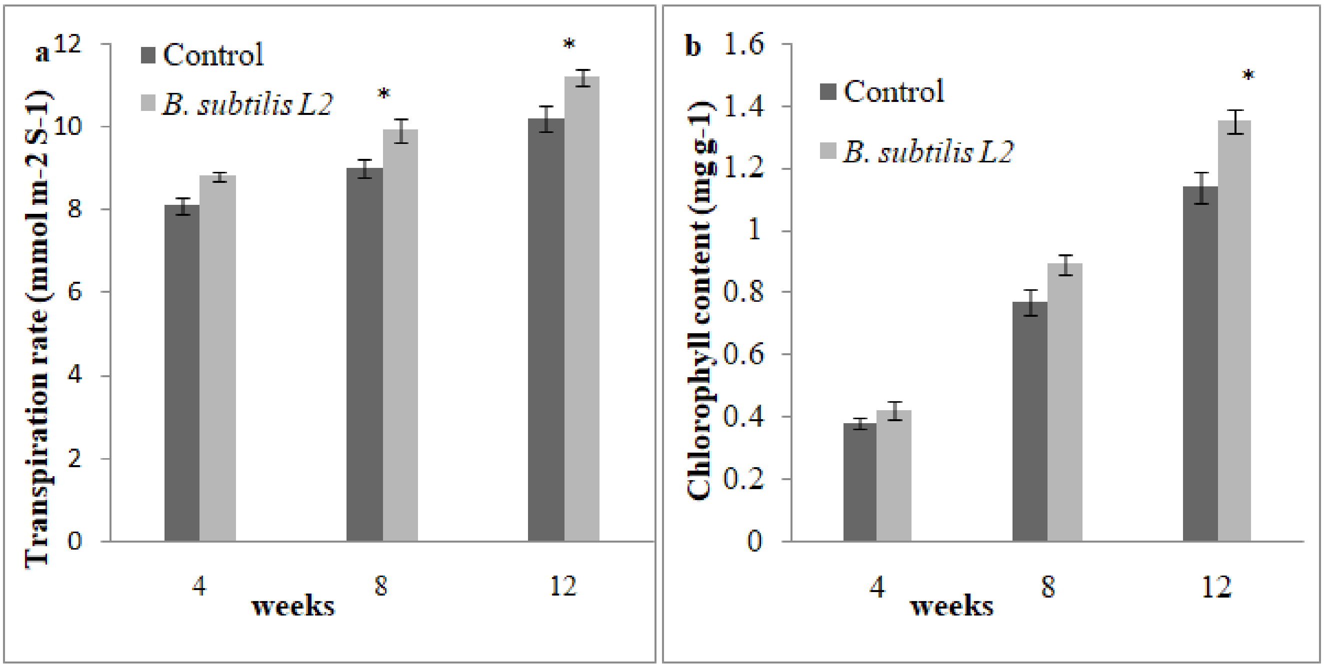

Fig. 2. Effect of inoculation with *B. subtilis* L2 on transpiration rate (a) and chlorophyll content (b) of Ginger (*Zingiber officinale*).

subtilis L2 has shown significant results for plant growth-promoting characteristics such as IAA, phosphate solubilisation and siderophore production (Table 1). Several researchers (46) reported the production of siderophore and phytohormones such as IAA and gibberellins in *Pseudomonas* sp., *Rhizobium* sp. and *Azotobacter* sp. isolated from the sugarcane field rhizosphere. The production of phytohormones, enzymes antagonistic activity in endophytic PGPR strains (47). Some authors (19) recorded the production of the rich quantity of siderophore in *P. fluorescence* NCIM 5096 and *P. putida* NCIM2847.

Our results showed that at 8 and 12 weeks after planting (WAP) inoculation with *B. subtilis* L2 significantly improved height of the plant, length of leaf and the number of leaves per plant and leaf width of ginger compared to control. There are many studies which showed that inoculation with rhizobacteria increases the plant, height, plant biomass and plant growth of legume crops (18, 28, 33, 43, 44). Inoculation with *B. subtilis* PTS-394 increased plant growth of tomato compared to control (38). In a study it was confirmed that the inoculation of effective *B. japonicum* strain significantly increased the plant growth of soybean (27).

The results showed that decrease of leaf transpiration and chlorophyll content as compared to the control at 4 WAP, respectively (Fig. 2a, b). Uninoculated control, the transpiration rate and the chlorophyll content are primarily reduced at 4 WAP. There are reports stating that control (at 180 days after planting) increase chlorophyll content compared to 120 days after planting (48). The results of this study indicated that the inoculation of *B. subtilis* L2 in the ginger plant could influence the transpiration rate and the chlorophyll content at 8 and 12 WAP. Similarly, promotion in plant growth promoting rhizobacteria has been reported improving transpiration and chlorophyll content in mungbean (49). Plant growth-promoting rhizobacteria inoculation increased chlorophyll content in legume crops such as mungbean, pea and soybean (49–51).

Conclusion

Bacilus subtilis had a significantly positive impact on plant growth, transpiration rate and chlorophyll content of ginger (*Zingiber officinale*). At 12 weeks after planting has substantial plant growth indicated by the significantly increased plant growth parameters, transpiration rate and chlorophyll content. Inoculation with *B. subtilis* L2 enhanced plant height, leaf number, leaf length, transpiration leaf and chlorophyll content. It is concluded that there is a significant and positive effect of inoculation with *B. subtilis* L2 on growth and physiological properties of ginger. Inoculation of *B. subtilis* could play an important role in sustainable agriculture by improving plant growth and productivity besides playing important role in P-solubilization, IAA (Indole-3-acetic acid) and siderophore production. *B. subtilis* L2 strain can be used as a potential agent or biofertiliser for stimulation of ginger growth and yield.

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Authors' contributions

DJ designed the experiments and wrote the manuscript. KA and AA interpreted the data and corrected the manuscript. YE performed analysis. KS and DK performed the methodology. All authors have read and approved the paper.

Conflict of interests

Authors do not have any conflict of interest to declare.

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