RESEARCH ARTICLE

Enhancement of Arabidopsis Growth with Inoculation of Phosphate-solubilizing and Nitrogen-fixing Bacteria

Yuri Lee

North London Collegiate School, 33 Global Edu-ro 145 beongil Daejeongeup, Seogwiposi, Jeju-do, 63644, South Korea; yurilee0408@gmail.com

ABSTRACT: The enhanced application of chemical fertilizers and pesticides not only has a limitation on growing crops but also has a negative impact on the environment. To improve these disadvantages, the plant growth-promoting bacteria (PGPB), *Pseudomonas fluorescens*, and *Bradyrhizobium japonicum* on the growth of Arabidopsis was examined. The inoculation of both bacteria has the main function of root protection, phosphate solubilization, and nitrogen-fixing. Six different inoculation conditions were tested using two types of bacteria: PF 1X, PF 100X, BJ 1X, BJ 100X, PF+BJ 1X, and PF+BJ 100X. Both bacteria assays aimed to address and measure the effects on plant development under normal or drought stress conditions. The growth and mass of Arabidopsis were significantly enhanced after the inoculation of plant growth-promoting bacteria in both conditions that were tested. This showed plant growth via improvement of soil health or through alleviating stress conditions. The results suggest that plant growth-promoting bacteria can accelerate and increase the growth and mass of plants, possibly by the fixation of nitrogen and by solubilizing phosphate, leading to the potential advantage of PGPB inoculation for sustainable agriculture.

KEYWORDS: Plant Biology; Bacteria; Nitrogen Fixation; Phosphate Solubilizing; Sustainable agriculture.

Introduction

Sustainable agriculture is farming in sustainable ways, which means meeting society's present food needs, without compromising the ability of future generations to meet their needs. Sustainable agriculture seeks to integrate three main objectives into its practices: a healthy environment, economic profitability, and social and economic equity. Several key sustainable farming practices have been developed—for example: rotating crops and planting a variety of crops; applying mechanical or biological pest controls; integrating livestock and crops, etc.[1]

Improved crop productivity and enhanced food security with a reduced or negligible application of chemical fertilizers and pesticides have become a major concern these days. Plant growth-promoting bacteria (PGPB) may be one of the keys to such sustainable agricultural practices. PGPB can serve the twin purpose of reducing chemical fertilizers and improving soil health.[2] Among the many PGPB, *Pseudomonas fluorescens*, a phosphate solubilizing bacteria, is known to protect the roots of some plant species against parasitic fungi as well as some plant-eating nematodes.[3] *Bradyrhizobium japonicum* is a species of legume-root nodulating, micro-symbiotic nitrogen-fixing bacteria.[4] The species is one of many Gram-negative, rod-shaped bacteria commonly referred to as *rhizobia*. *B. japonicum* is often added to legume seeds to improve crop yields.

There are common scientific model plants. *Arabidopsis* is a genus in the family Brassicaceae. They are small flowering plants related to cabbage and mustard. It is one of the model organisms used for studying plant biology and the first plant to have its entire genome sequenced. Phenotypic changes in Arabidopsis are easily observed, making it a very useful model.[5]

It was expected that PGPB inoculated Arabidopsis may enhance growth compared to non-inoculated Arabidopsis. Previous studies indicated that phosphate solubilization bacteria support water uptake under drought stress. Also, nitrogen fixation may be inhibited by drought stress. Therefore, it was hypothesized that the plants with drought stress may show larger differences in plant growth between PDPB inoculated and non-inoculated plants.

Methods

Plant growth conditions:

Sterilized seeds were sewn onto moist soil (autoclaved) and stratified at 4oC for 3 days. The growth response of plants in the soil were evaluated by fresh weight under controlled environmental conditions (constitutive light, 150 mmol m22 s21 light intensity, 75% relative humidity). Drought stress was initiated 1 week after planting. Plants were watered every 7 or 8 days for the stress condition.

PGPB inoculant preparation:

For the PGPB inoculation, strains were grown in LB medium for 18 hours at 28 °C with continuous shaking at 200 rpm, and the cells were collected by centrifugation at 6,000 rpm for 10 minutes, washed, and re-centrifuged in sterile distilled water to obtain a bacteria inoculum of approximately 10^9 cfu/mL.

PGPB application to plants and drought stress:

Three different dilution series (1X; 10X; 100X) of the bacterial suspensions (10 mL/pot) were sprayed onto the seedlings on the soil. For the uninoculated control, an equal volume of sterile water was added. Drought stress was initiated one week after planting. Plants were watered every 7 or 8 days for the stress condition.

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Data analysis:

Every two weeks after the inoculation treatment, ten plants were harvested at random from each pot. The root and above-ground tissue fresh weights were measured to determine if any biomass changes. To test for any changes in stress tolerance, the marker gene expression was measured accordingly as well.

Results and Discussion

Preparation of Arabidopsis and PGPB:

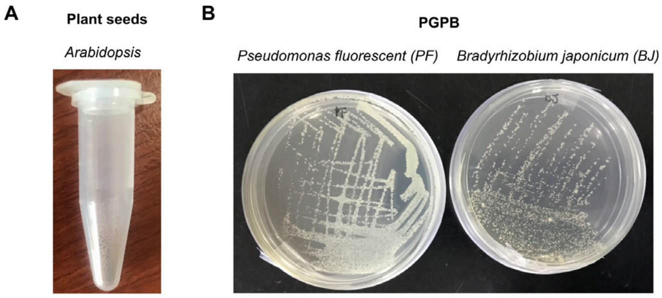

Figure 1: Seeds of Arabidopsis thaliana in test tubes and the bacteria used in this study. (A) Arabidopsis seed (B) (left) *Pseudomonas fluorescent*, (right) *Bradyrhizobium japonicum*.

Arabidopsis (rockcress) is a genus in the family Brassicaceae. They are small flowering plants related to cabbage and mustard. It is one of the model organisms used for studying plant biology and the first plant to have its entire genome sequenced.[5] Arabidopsis was selected as the first model for testing the effect of PGPB inoculation on plant growth because it is easy to observe the phenotypic changes in Arabidopsis, and it has a short life span. (Figure 1)

P. fluorescens is a common gram-negative, rod-shaped bacterium. It is known to protect the roots of some plant species against parasitic fungi as well as some plant-eating nematodes. Also, it solubilizes phosphate to help increase the absorptive surface of plant roots for the uptake of water and nutrients. *B. japonicum* is a symbiotic nitrogen-fixing soil bacterium that has the ability to form root nodules.

P. fluorescens and *B. japonicum* are good inoculant candidates because they colonize roots and create a favorable environment for development and function. The success and efficiency of PGPB as inoculants for agricultural crops are influenced by various factors, among which the ability of these bacteria to colonize plant roots, the exudation by plant roots, and the soil health.[6] *P. fluorescens* was used to suppress plant diseases by protecting the seeds and roots from fungal infection. It also solubilizes inorganic phosphorus into soluble phosphate for plants to uptake and manages the optimum level of soil phosphorus for crop production. This experiment was focused on comparing the growth of plants in two conditions (normal conditions and drought stress conditions). Previous research indicated that *B. japonicum* enhances survival under conditions of salinity stress.[7] Moreover, it is known as a nitrogen-fixing bacteria. They convert nitrogen in the atmosphere into ammonium (NH_4^+) and nitrates that plants can absorb through their roots which will increase the growth rate.

Based on previous research on plant growth-promoting bacteria, the following hypotheses can be made. There will be a significant change in the biomass of plants between inoculated and non-inoculated. In addition, the change will be notable when *B. japonicum* is inoculated or not because its purpose is to survive in a stressed condition, which is the independent variable in our experiment.

Imaging PGPB Cell Morphology on Brightfield Microscope:

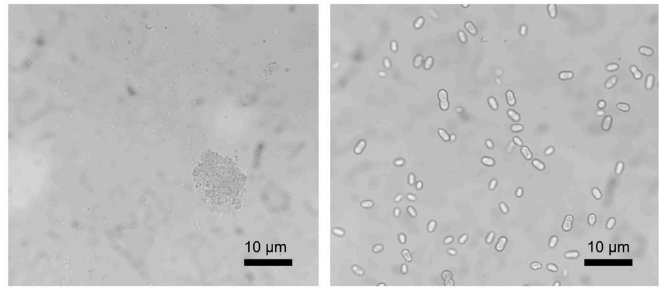

Figure 2: The 40 X microscopic image of *Pseudomonas fluorescens* (left) and *Bradyrhizobium japonicum* (right). Scale bar: 10 μm.

To observe the phenotypic characteristics of the PGPB bacteria, images using a light microscope were taken. The size of the *P. fluorescens* was relatively smaller than *B. japonicum* and it was difficult to observe (Figure 2, left). The image showed that *P. fluorescens* is rod-shaped and able to aggregate together. However, the image of *B. japonicum* shows the clear shape of the bacteria, which is relatively larger than *P. fluorescens*. *B. japonicum* has a rod-shaped body. The microscopic image indicates that these bacteria are not cross-contaminated. Therefore, it was ready to be used for inoculation with Arabidopsis (Figure 2, right).

Germination of Arabidopsis and plant maintenance:

Figure 3: Image of germinated Arabidopsis on a petri dish a week after seeding.

After 30 to 50 Arabidopsis were seeded on the Petri dish, Arabidopsis was successfully germinated on a Petri dish plate. The germinated Arabidopsis shows 4-5 green cotyledons from each plant. The length of the roots was about 2-3 cm (Figure 3). The germinated Arabidopsis were then carefully moved to the soil. After the germination plant incubator used in this study. The condition was set to 16 hours light and 8 hours dark. 10 am to 2 pm was set to 22 °C. 2 pm to 10 am was set to 18 °C.

After germination, Arabidopsis grew well in the control environment. The image of Arabidopsis was captured after two weeks of growth (Figure 4, left) and three weeks of growth (Figure 4, right). As Arabidopsis grew, the number of leaves

Figure 4: The image of Arabidopsis after two weeks of growth (left) and three weeks of growth (right).

increased and became bigger. Arabidopsis were prepared for two conditions: water was supplied three times a week (normal condition) and water was supplied only once a week (drought stress). Then seven different conditions for bacteria inoculation were prepared for each water supply condition: Mock, PF 1x, PF 100x, BJ 1x, BJ 100x, PF+BJ 1x, and PF+BJ 100x (the abbreviation of bacteria was used, 1x and 100x indicates the concentration of bacteria). 100x means 100 times of 1x bacteria condition was inoculated in Arabidopsis.

Measuring the weight of the plants to analyze the growth rate:

Figure 5: The weight of *Arabidopsis thaliana* after 5 weeks was measured with a densitometer. The whole Arabidopsis without stem and roots were collected (left). Densitometer was used to measure the weight of the collected Arabidopsis (right).

After 6 weeks after the germination of Arabidopsis, the whole plant without the root and stem was collected on the petri dish as shown in Figure 5, left. Then, the collected leaves were placed on the densitometer and their weight was measured (Figure 5, right). For each condition, three individual samples were collected, and the weight was measured.

The plant weight change on normal water conditions (three times a week) and drought stress conditions (once a week:

After the weight was measured for each condition, the average plant weight was analyzed in Figure 6. Interestingly, the plant weight was increased only when BJ 100x was inoculated in normal water conditions. This result indicates that when water is sufficiently provided, *B. japonicum* supports the growth of the Arabidopsis (Figure 6, left). Since *B. japonicum*

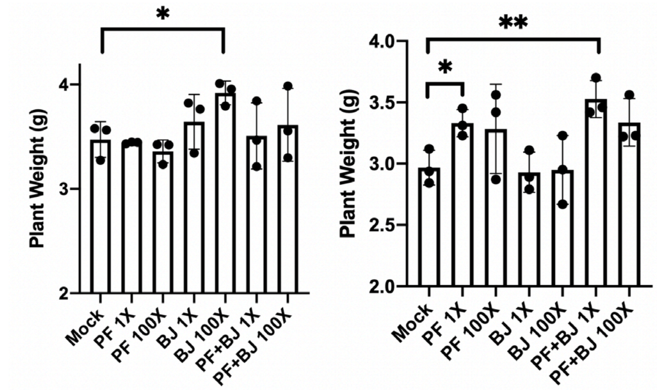

Figure 6: Bar graph of plant weight in normal water condition (left) and in drought stress condition (right). Triplicate samples were prepared. Each measurement was presented as dark circle dots on the graph. The mean and standard deviation were described in the graph. PF = only *Pseudomonas fluorescens* was inoculated. BJ = only *Bradyrhizobium japonicum* was inoculated. PF+BJ = both bacteria were inoculated. 1x and 100x indicates the concentration of bacteria inoculated. Student t-test, $p < 0.05$ (*) and $p < 0.01$ (**).

is known to be a nitrogen-fixing bacteria, the growth of the Arabidopsis may be enhanced by the extra nitrogen supplied by this bacterium.[7] Previous research also indicates that nitrogen is part of the chlorophyll molecule, which supports the plants to sustain their green color and supports photosynthesis. Therefore, a sufficient nitrogen supply is essential for plant growth. However, when the Arabidopsis was grown in drought stress conditions, inoculating with *B. japonicum* alone was not sufficient to enhance the growth of the Arabidopsis. Instead, *P. fluorescens* alone and a combination of both bacteria significantly increases the growth of the Arabidopsis (Figure 6, right). *P. fluorescens* has two important functions: supports water supply for plants and solubilizes phosphate. Since *P. fluorescens* only increases the weight of the Arabidopsis in drought stress conditions, the water supplying function of this bacteria may greatly influence the growth of the Arabidopsis. Moreover, previous papers indicate that p-solubilizing bacteria alleviate drought stress and promote plant growth.[8]

Conclusion

As the human population continues to increase, the demand for food may escalate. Seven decades ago, the Green Revolution increased agricultural production globally with the development of chemical fertilizer. It saved about a billion people from starvation and undernourishment. However, intensive agriculture, crop monoculture, and the use of extensive chemical fertilizers disturb the ecosystem. Considering the positive impact of plant growth-promoting bacteria (PGPB) on bio-fertilization, biocontrol, and bioremediation, PGPB deliver a positive influence on crop productivity and the ecosystem.

In this research, two possible PGPB, *P. fluorescens* and *B. japonicum*, were selected and their effect on the growth of the Arabidopsis were examined. The results indicate that the growth and mass of Arabidopsis were greatly enhanced after the inoculation of PGPB. *B. japonicum* enhances plant growth in normal water conditions. *P. fluorescens* increases plant growth in drought stress conditions. These results suggest that PGPB can accelerate and increase the growth and mass of the plant,

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possibly by the fixation of nitrogen and solubilizing phosphate, leading to the potential advantage of PGPB inoculation for sustainable agriculture. In conclusion, the selection of a particular strain is most important in obtaining the maximum efficiency in terms of improved plant growth and development.

In future studies, PGPB (*P. fluorescens and B. japonicum*) will be grown under the same condition before it is inoculated to the plants. Solanum Lycopersicum cv. Micro-Tom and Bok choy which are easily found in agriculture will be used as another experimental plant. The growth response of plants in the soil will be evaluated by fresh weight under identical environmental conditions as Arabidopsis (constitutive light, 150 mmol m22 s21 light intensity, 75% relative humidity). PGPB will be further used in an efficient way for sustainable agriculture. The bacteria will be inoculated in a 1:1 ratio of *P. fluorescens* and *B. japonicum* bacterial suspensions with agriculture products in drought stress conditions.

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Author

Yuri Lee is a year 11 student currently in NLCS Jeju. She is part of various sports squads such as basketball, football, volleyball, and netball. Yuri demonstrates aptitude in science, especially for biological science. She won an award in the Genius Olympiad and KSCY with her biology research project.