

Suitable Materials for *Paenibacillus* sp. BSR₁₋₁ Immobilization and Crop Growth Stimulation under Low Water Condition

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ABSTRACT

Agricultural challenges due to a water shortage are factors limiting plant growth and productivity worldwide. One way to improve plant growth under unsuitable conditions is to use plant growth-promoting bacteria (PGPB). The objective of this study was to investigate the ability of PGPB to increase peanut, rice, and sweet corn growth under low water conditions. Suitable agricultural materials were selected first to be used in *Paenibacillus* sp. BSR₁₋₁ immobilization. The materials were water hyacinth, reed, and coconut husk. Water hyacinth maintained the bacterial cell number when kept at either -4, 4, or 27-30 °C for both storage times, and water hyacinth soaked with a bacterial cell suspension prepared in 0.5 % ammonium sulfate ((NH₄)₂SO₄) + 1 % glucose was the most suitable method to immobilize the bacterial cells. *Paenibacillus* sp. BSR₁₋₁ with indole-3-acetic acid (IAA) and exopolysaccharide-producing abilities significantly increased root growth of peanuts under the low water condition.

Root length and dry weight of inoculated peanut grown under low water conditions were 138.91 % and 156.51 % higher than uninoculated peanut, respectively. This bacterial isolate significantly increased rice shoot dry weight and root length under low and full water conditions. However, it only increased shoot length and root dry weight under the full water condition. *Paenibacillus* sp. BSR₁₋₁ increased the dry

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weight of sweet corn under both conditions but only increased the root length of sweet corn under the full water condition. The shoot dry weight of inoculated sweet corn under the low water condition was 170.59 % higher than that of the un-inoculated sweet corn. When rice received *Paenibacillus* sp. BSR₁₋₁ under the full water condition, and when peanuts received these bacteria under both conditions, they could produce more tillers and pods than the un-inoculated plants. Thus, *Paenibacillus* sp. BSR₁₋₁ was an appropriate strain to use as a biofertilizer for agricultural proposes in water-limited areas.

Keywords: Corn, low water, *Paenibacillus*, peanut, plant growth-promoting bacteria, rice

INTRODUCTION

Drought exerts negative impacts on plant growth and yield in several ways, including decreasing the water-soluble nutrient diffusion to the plant root inducing oxidative stress in plants, which results in lipid peroxidation, membrane degradation, and protein degradation (Vurukonda et al., 2016). There are several suggestions to mitigate the adverse effects of drought on plant growth and yield, including using water-saving irrigation, short-cycle, and drought-tolerant plants, traditional breeding, and drought-tolerant transgenic plants (Food and Agriculture Organization of the United Nations [FAO], n.d.; Niu et al., 2018). Moreover, the application of PGPB is another means to stimulate the growth of plants under limited water (Niu et al.,

2018). Important characteristics of PGPB to support plant growth include nitrogen fixation, phosphate solubilization, ACC deaminase activity, siderophore production, and plant growth regulator production (de Souza et al., 2015). There have been several research reports on the successful use of PGPB under low water conditions. For example, inoculation of *Zea mays* seed with exopolysaccharide-producing bacteria (*Pseudomonas aeruginosa* (Pa2)) could increase protein and sugar concentrations and decrease the activity of antioxidant enzymes in leaves under stress conditions (Naseem & Bano, 2014). Inoculations of lettuce (*Lactuca sativa*) with *Bacillus megaterium* TV 6D (B1) and *Bacillus subtilis* TV 12H (B2) significantly increased the plant growth, yield, and nutrient content grown under lower irrigation levels (Sahin et al., 2015). Also, the seed germination and seedling growth of foxtail millet (*Setaria italica*) inoculated with *Pseudomonas fluorescens* DR7, which could produce ACC deaminase and exopolysaccharide, were increased under drought stress because the moisture increased in inoculated soil (Niu et al., 2018). Moreover, inoculation of peanut shoots with *Bradyrhizobium* strain ESA 123 increased the number of nodules and activation of metabolic gene expression for plant protection under water deficit stress (Brito et al., 2019).

Paenibacillus sp. BSR₁₋₁ with the ability to produce IAA (Somtrakoon et al., 2019), ammonia, exopolysaccharide, and drought tolerance has been reported to stimulate the root growth of aquatic morning glory in our

previous study (Somtrakoon et al., 2022), which was used as a model of PGPB in this study. However, successful use of PGPB depends on their survival ability, as they need to compete with indigenous bacteria and settle around the root zone (de Souza et al., 2015). Using immobilized microbial cells is expected to overcome the limiting factors that restrict the use of PGPB in agricultural soil. Several advantages of using immobilized microbial cells have been reported, including maintaining high microbial biomass, preserving high microbial activity, the resistance of microbial cells to toxic chemicals, and providing long cellular viability (Bashan, 1998; Martins et al., 2013; Santos et al., 2019).

Several immobilization materials, including agar, sodium alginate, hydrogel, and composite materials, have been used as immobilized cell carriers for biochemical production and wastewater treatment (Lu et al., 2020; Martins et al., 2013). The possible mechanisms of immobilization technologies include adsorption onto the surface of immobilized materials, encapsulation in immobilized materials, entrapment within immobilization materials, and containment within a polymer (Lu et al., 2020). Important criteria for immobilized materials include insoluble, non-toxic, high stability, high diffusivity, easy immobilization process, high biomass retention, and cheap (Martins et al., 2013). Based on these suitable criteria for immobilized materials, agricultural residues can be used as carriers for inoculating microorganisms into agricultural soil. The benefits of carriers from agricultural residues

are that they are environmentally friendly, easy to apply, provide high porosity, provide a high surface area for cell attachment and nutrient transfer (Kirdponpattara et al., 2021; Santos et al., 2019). Examples of natural carriers from agricultural residues include water hyacinth (Kirdponpattara et al., 2021), coconut husk, sawdust, and rice straw (Somtrakoon et al., 2022). The objectives of this study were to find suitable natural carriers for immobilization of *Paenibacillus* sp. BSR₁₋₁ and to investigate the ability of *Paenibacillus* sp. BSR₁₋₁ to stimulate the growth of peanut cultivar 'Tainan 9' (*Arachis hypogaea*), sweet corn (*Zea mays* var. *saccharata*), and rice cultivar 'KDML 105' (*Oryza sativa*) when cultivated under full and low water conditions.

MATERIALS AND METHODS

Plant Growth-Promoting Activity

Paenibacillus sp. BSR₁₋₁ was previously isolated from a paddy field in Wapi Pathum District, Maha Sarakham Province, Thailand, by Assoc. Prof. Aphidech Sangdee. It had 97 % similarity to *Paenibacillus polymyxa* based on a 16s rDNA sequence. These bacteria were cultured in nutrient agar and a 24 h culture of *Paenibacillus* sp. BSR₁₋₁ was used as an inoculum to test the promoting plant growth. Further plant growth-promoting activities were tested in this study, including ACC deaminase production, siderophore production, and potassium solubilization. ACC deaminase activity was screened by the method described in Penrose and Glick (2003). Potassium solubilization activity was

tested according to the method described in Prajapati and Modi (2012). Siderophore production was tested according to the methods described in Pérez-Miranda et al. (2007). Finally, carboxymethyl cellulose degradation was tested by the methods described in George et al. (2001).

Suitable Preparation of Immobilized Cells in Agricultural Residues

Agricultural residues, including coconut husk, reed, and water hyacinth, were cut into 1x1 cm pieces and autoclaved at 121 °C for 15 min. Immobilized *Paenibacillus* sp. BSR₁₋₁ cells in agricultural residues were prepared by soaking these agricultural residues with a cell suspension of *Paenibacillus* sp. BSR₁₋₁ prepared in 0.85 % sodium chloride (NaCl) for 3 h. Cells of *Paenibacillus* sp. BSR₁₋₁ immobilized in each agricultural residue was kept at -4 °C, 4 °C, and 27-30 °C for 10 and 30 days. *Paenibacillus* sp. BSR₁₋₁ cells were counted after being kept immobilized for 10 and 30 days (Table 1).

The suitable ammonium sulfate and glucose concentrations for immobilized *Paenibacillus* sp. BSR₁₋₁ in each agricultural residue were tested. Each agricultural residue was soaked in a cell suspension of *Paenibacillus* sp. and prepared with three formulations of ammonium sulfate and glucose (0.5 % ammonium sulfate + 1 % glucose, 1 % ammonium sulfate + 2 % glucose, and 1.5 % ammonium sulfate + 3 % glucose) for 3 h. The initial number of *Paenibacillus* sp. BSR₁₋₁ in each agricultural residue was counted

after the immobilization process (Table 2). Then, the cells of *Paenibacillus* sp. BSR₁₋₁ immobilized in each agricultural residue was kept at 4 °C for 10 and 30 days. The number of *Paenibacillus* sp. BSR₁₋₁ has counted again on days 10 and 30 after preparation. The best formulation of ammonium sulfate and glucose for maintaining cells of *Paenibacillus* sp. BSR₁₋₁ was sent to analyze the carbon and nitrogen ratio at the Central Laboratory (Thailand) Company Limited, Khonkaen Province.

Pot Experiment

Water hyacinth was soaked in a cell suspension of *Paenibacillus* sp. BSR₁₋₁ prepared in 0.5 % ammonium sulfate + 1 % glucose to prepare immobilized cells. Then, the immobilized cells of *Paenibacillus* sp. BSR₁₋₁ were used to stimulate the growth of peanut, rice, and sweet corn in a pot experiment. Seeds of peanut cultivar 'Tainan 9', rice cultivar 'KDML105', and sweet corn were received from a farmer in Chiangmai Sub-District, Pho-Chai District, Roi-Et Province, Thailand.

Soil from Donnong Village, Kham Riang Sub-District, Kantharawichai District, Maha Sarakham Province, Thailand, was collected for use in this study. The soil characteristics, including pH, organic matter, soil texture, available phosphorus, exchangeable potassium, exchangeable calcium, and exchangeable magnesium, at the beginning and the end of the experiment were determined via analysis at Soil-Fertilizer-Environment Scientific Development Project, Department of Soil

Science, Faculty of Agriculture, Kasetsart University, Thailand. The soil used in the pot experiment was prepared by autoclaving and divided into pots for planting the peanut, rice, and sweet corn. Soil moisture contents on days 1–4 after soaking the soil at room temperature were 13.07 ± 0.23 %, 8.84 ± 0.29 %, 7.66 ± 0.73 %, and 2.67 ± 0.79 %, respectively. The experimental pots for each plant were laid out in a completely randomized design with one factor. There were four treatments in this study: 1) uninoculated control at low water, 2) uninoculated control at full water, 3) inoculation of immobilized *Paenibacillus* sp. BSR₁₋₁ at low water, and 4) inoculation of immobilized *Paenibacillus* sp. BSR₁₋₁ at full water. Each treatment was performed as ten replicates. There were some differences between plant species, as described below.

Peanut. A total of 1.5 kg of the autoclaved soil was poured into each 24.13 cm diameter pot. Peanut seeds were submerged in distilled water for 48 h. Then, five germinated peanut seeds were added to each experimental pot. After seedling emergence, only one seedling of 12-day-old peanut with comparable sizes in each pot was left to grow. Then, 10 g of water hyacinth with immobilized cells of *Paenibacillus* sp. BSR₁₋₁ was spread on the surface of the soil on day 30. The initial concentration of *Paenibacillus* sp. BSR₁₋₁ immobilized in water hyacinth was 7.76 ± 0.34 log cfu/g. Free cells of *Paenibacillus* sp. BSR₁₋₁ with an initial concentration of 7.22 ± 0.06 and 7.88 ± 0.07 log cfu/ml were re-inoculated onto water hyacinth

in the experiment pot on days 62 and 92, respectively. The irrigation of peanuts was divided into three phases. Firstly, water with 30 ml of water every day until day 30 of the experiment. The second phase started after the first inoculation of *Paenibacillus* sp. BSR₁₋₁ immobilized in water hyacinth and watered with 50 ml of water to each experimental pot every day for full water and every four days under the low water condition. The third phase of irrigation began when the peanut was 50 days old, and the irrigation of the low water peanut was changed to every other day until the end of the experiment. After flowering, more soil was poured around each peanut shoot in each pot experiment.

Rice. An amount of 1.25 kg of the autoclaved soil was poured into each experimental pot with 27.94 cm diameter. Rice seeds were immersed in distilled water for 48 h and transferred to the experimental pots, with each pot containing 15 seeds. After 12 days, the rice seedlings were thinned to 10 seedlings in each experimental pot. For the first 30 days of the experiment, the pots were watered every day. After that, water was poured into the rice pots under full water conditions until the water level was 5 cm above the soil surface. After that, rice planted under the low water condition was watered with 100 ml of water every four days. On day 50 of the experiment, only the low water pot was changed to 100 ml of water every day. Then, 10 g of water hyacinth with immobilized cells of *Paenibacillus* sp. BSR₁₋₁ was spread on the

soil surface on day 35 of the experiment under full and low water conditions. The initial cells of *Paenibacillus* sp. were at 7.76 ± 0.34 log cfu/g. Then, 15 ml of free cells of *Paenibacillus* sp. (7.22 ± 0.06 log cfu/ml) were re-inoculation onto water hyacinth in the experimental pots on day 62.

Sweet Corn. Sweet corn seeds were soaked in distilled water for 48 h. Then, five emerged seeds were inoculation into each experimental pot with a diameter of 15.24 cm containing 750 g of soil and thinned to one plant per pot on day 12. On day 30, 5 g of water hyacinth with immobilized cells of *Paenibacillus* sp. BSR₁₋₁ was spread on the soil surface. The initial cell number of *Paenibacillus* sp. BSR₁₋₁ in water hyacinth was 7.76 ± 0.34 log cfu/g. Each pot was watered every day until day 30 of the experiment. After that, 20 ml of water was poured into the experimental pots every day for the full water condition, and the schedule of watering was four days under the low water condition. After sweet corn was 50 days old, the irrigation pattern for the low water condition was changed to every other day until the end of the experiment. No chemical fertilizer was applied to the experimental pots because of only the effect of *Paenibacillus* sp. BSR₁₋₁ on the plant's growth was investigated. At the end of the experiment, 107-day-old peanuts, 78-day-old rice, and 65-day-old sweet corn were collected to analyze the plant growth parameters (root length, shoot length, number of leaves, shoot and root dry weight, and chlorophyll content in leaves). Chlorophyll content measurement was done

according to Huang et al. (2004) for all plant leaves. Two pots of rice in low water condition when receiving *Paenibacillus* sp. BSR₁₋₁ were left until 100 days old to observe tiller and grain production.

Statistical Analysis

Data in Tables 1, 2, 3, and 4 are expressed as mean \pm standard error (SE). A one-way analysis of variance (ANOVA) was used for plant growth analysis, and two-way ANOVA was used for variance analysis for bacterial survival. The least-square difference (LSD) was used for the pairwise comparison of all experiments.

RESULTS AND DISCUSSION

The plant growth-promoting bacteria used in this study, *Paenibacillus* sp. BSR₁₋₁, showed several abilities, such as IAA, exopolysaccharide and ammonia production, drought tolerance (Somtrakoon et al., 2019, 2022), and carboxy methyl cellulose degradation. However, this bacterial isolate could not solubilize phosphate (Somtrakoon et al., 2019) and potassium, and it could not produce siderophores and ACC deaminase. Our previous study indicated that the cells of *Paenibacillus* sp. BSR₁₋₁ immobilized in sawdust, rice straw, and coconut husk could induce aquatic morning glory root growth under drought conditions (Somtrakoon et al., 2022). Thus, this study was undertaken to determine more suitable agricultural materials and a suitable ratio for ammonium sulfate and glucose when preparing cell suspensions of *Paenibacillus* sp. BSR₁₋₁ for immobilized microbial cells. Coconut

husk has been used in a previous study (Somtrakoon et al., 2022) that also tested together with other agricultural residues, including reed and water hyacinth.

The results revealed that the most suitable agricultural residue to immobilize *Paenibacillus* sp. BSR₁₋₁ was water hyacinth. The results in Table 1 indicate that water hyacinth could maintain the cell number of *Paenibacillus* sp. BSR₁₋₁ when kept at either -4, 4, or 27–30 °C. The cell numbers of *Paenibacillus* sp. BSR₁₋₁ immobilized in water hyacinth were not significantly different when kept at different temperatures (-4, 4, or 27–30 °C) and storage times (10 and 30 days). The number of *Paenibacillus* sp. BSR₁₋₁ cells on day 30 were 7.61–9.02, 6.69–6.87, and 9.15–9.34 log cfu/g when immobilized in the reed, coconut husk, and water hyacinth, respectively. Moreover, 0.5% ammonium sulfate and 1% glucose were the suitable concentrations of the nutrients for preparing cell suspensions of *Paenibacillus* sp. BSR₁₋₁ immobilized in the reed, coconut husk, and water hyacinth. After storage for 30 days at 4 °C, the cell numbers of *Paenibacillus* sp. BSR₁₋₁ immobilized in water hyacinth were 7.85 log cfu/g, which was significantly higher than that immobilized in reed (6.83 log cfu/g) and coconut husk (5.47 log cfu/g) (Table 2).

Thus, water hyacinth with 0.5% ammonium sulfate and 1% glucose was used as the agricultural material and solution to prepare the cell suspension of *Paenibacillus* sp. BSR₁₋₁ for the pot experiment. Based on the results from Table 2, the significant difference in cell number for each agricultural

material at the beginning (Day 0) might depend on the sorption capacity of each agricultural material for the bacterial cells. A major factor that limited the successful use of microbial inoculants was a low cell number and low bacterial activity after introducing free cells to soil with biotic and abiotic stress in the environment (Partovinia & Rasekh, 2018). Thus, cell immobilization in water hyacinth was used to carry the cells of *Paenibacillus* sp. BSR₁₋₁ to the planted soil in this study. Immobilizing microbial cells in agricultural residues was expected to protect the microbial cells from environmental stress, thereby increasing microbial cell stability and density (Kirdponpattara et al., 2021). The main characteristic of the suitable carrier should be nontoxic to microbial cells (Yao et al., 2011). This study revealed that water hyacinth was the most suitable agricultural residue for immobilization of *Paenibacillus* sp. BSR₁₋₁ cells due to this material being able to maintain the microbial cell number at all storage temperatures. The aerenchyma tissue in water hyacinth has high porosity and a high ability to absorb water, which is useful for nutrient transfer and cell adsorption (Kirdponpattara et al., 2021). Moreover, the characteristics of the carrier surface may affect the microbial absorption onto them. A study by Kirdponpattara et al. (2021) reported that water hyacinth could immobilize yeast cells more than cocoon because the yeast cell had a high affinity to the water hyacinth surface than the other. Microbial cell absorption on carriers with smooth surfaces is difficult (Kirdponpattara et al., 2021).

Table 1
Effect of storage temperature on cell numbers of *Paenibacillus* sp. BSR₁₋₁ immobilized in each agricultural material

Immobilized materials	- 4 °C	4 °C	27 - 30 °C
<u>Day 10</u>			
Water hyacinth	9.27±0.036aA	9.35±0.004aA	9.13±0.044aA
Reed	8.63±0.029bA	8.18±0.088bA	8.27±0.110bA
Coconut husk	6.02±0.020cA	6.67±0.104cA	6.69±0.078cA
Material	**		
Temperature	ns		
Material x Temperature	**		
<u>Day 30</u>			
Water hyacinth	9.34±0.018aA	9.15±0.157aA	9.16±0.038aA
Reed	8.57±0.410bA	9.02±0.276aA	7.61±0.230bB
Coconut husk	6.78±0.094cA	6.87±0.176bA	6.69±0.106cA
Material	**		
Temperature	*		
Material x Temperature	*		

Note. Different lower-case letters show significant differences between agricultural residues for the same temperature, and different capital letters show significant differences between temperatures for the same agricultural residues. Symbols: ns, *, ** denote non-significance ($P>0.05$), statistical significance ($P<0.05$), and high statistical significance ($P<0.01$), respectively. The number of bacterial cells suspended at the beginning was approximate 10^8 - 10^9 cfu/ ml (optical density of bacterial suspension at a wavelength of 600 nm = 0.5)

The carbon and nitrogen ratio of water hyacinth used in this study was 104.38: 1. The carbon and nitrogen ratio of water hyacinth after soaking in the cell suspension of *Paenibacillus* sp. BSR₁₋₁ was prepared in 0.5% ammonium sulfate, and 1% glucose was 57.08: 1. In general, a carbon and nitrogen ratio of less than 20 has a chance to degrade nitrogen (Truong & Marschner, 2018). Meanwhile, nitrogen immobilization could occur at a C: N ratio of more than 20 (Truong & Marschner, 2018), and the values ranged between 20–30, indicating the suitability of this material for compost production (Wu et al., 2017). Thus, the water hyacinth used in this study with a carbon and nitrogen ratio greater than 30 is suitable as it is difficult to degrade after application to the soil as a bacterial cell carrier. The

addition of glucose and ammonium sulfate to water hyacinth did not change the carbon and nitrogen ratio, so it was optimum for composting. Thus, reuse of water hyacinth may be possible. Moreover, immobilized cells of *Paenibacillus* sp. BSR₁₋₁ can be stored at room temperature, and this is convenient when used in a real situation.

Growth of Economic Crops Under Low Water Condition

While immobilized *Paenibacillus* sp. BSR₁₋₁ was inoculated in soil, the dry shoot weight, dry root weight, and root length of rice KDML105 and sweet corn grown under both full and low water conditions were higher than that grown in soil without *Paenibacillus* sp. BSR₁₋₁ inoculation. The shoot dry weight, root dry weight, and

Table 2

Effect of glucose and ammonium sulfate concentration on cell numbers of *Paenibacillus* sp. BSR₁₋₁ in each immobilized material while kept at 4 °C

Immobilized materials	1% glucose + 0.5 % ammonium sulfate	2% glucose + 1.0% ammonium sulfate	3% glucose + 1.5% ammonium sulfate
<u>Day 0</u>			
Water hyacinth	9.06±0.05aA	5.76±0.12bC	6.21±0.04aB
Reed	6.33±0.05bB	7.24±0.19aA	6.14±0.10aB
Coconut husk	5.57±0.02cB	5.43±0.08cB	6.14±0.10aA
Material	**		
Nutrient	**		
Material x Nutrient	**		
<u>Day 10</u>			
Water hyacinth	8.59±0.40aA	6.27±0.17bB	6.53±0.23aB
Reed	6.50±0.41bB	7.69±0.11aA	6.37±0.04aB
Coconut husk	5.60±0.10bA	5.37±0.05cA	6.05±0.19aA
Material	**		
Nutrient	*		
Material x Nutrient	**		
<u>Day 30</u>			
Water hyacinth	7.85±0.11aA	5.59±0.13bB	5.93±0.04bB
Reed	6.83±0.08bAB	7.09±0.35aA	6.56±0.02aB
Coconut husk	5.47±0.04cA	5.48±0.17bA	5.91±0.02bA
Material	**		
Nutrient	**		
Material x Nutrient	**		

Note. Different lower-case letters show significant differences between agricultural residues for the same nutrient formulation, and different capital letters show significant differences between nutrients for the same agricultural residues. Symbols: * and ** denote statistical significance ($P < 0.05$) and highly statistical significance ($P < 0.01$), respectively. The number of bacterial cells suspended at the beginning was approximate $10^8 - 10^9$ cfu/ ml (optical density of bacterial suspension at a wavelength of 600 nm = 0.5)

root length of rice grown in the presence of *Paenibacillus* sp. BSR₁₋₁ in soil under both conditions were around 0.15–0.17 g, 0.05–0.07 g, and 16.4–17.4 cm, respectively. Meanwhile, the shoot dry weight, root dry weight, and root length of rice grown in the absence of *Paenibacillus* sp. BSR₁₋₁ in soil under both conditions were around 0.04–0.10 g, 0.03–0.03 g, and 8.3–9.0 cm, respectively. The shoot dry weight, root dry weight, and root length of sweet corn grown in the presence of *Paenibacillus* sp. BSR₁₋₁ in soil under both conditions were around

0.58–0.63 g, 0.17–0.21 g, and 26.75–34.33 cm, respectively. The shoot dry weight, root dry weight, and root length of sweet corn grown in the absence of *Paenibacillus* sp. BSR₁₋₁ in soil under both conditions were around 0.30–0.34 g, 0.08 - 0.09 g, and 20.06–20.29 cm, respectively (Table 3). Soil inoculated with immobilized *Paenibacillus* sp. BSR₁₋₁ also stimulated the growth of peanuts. The shoot length of peanut grown under low water conditions was shorter (28.42–30.72 cm) than that grown under full water conditions (39.42–44.49 cm);

however, the inoculation of *Paenibacillus* sp. BSR₁₋₁ increased the root dry weight and root length of peanuts grown under full and low water conditions. The root dry weight and root length of peanut grown in the presence of *Paenibacillus* sp. BSR₁₋₁ in soil under both conditions were around 0.25–0.36 g and 23.4–28.2 cm, respectively. Meanwhile, the root dry weight and root length of peanut grown without *Paenibacillus* sp. BSR₁₋₁ inoculation under both conditions were only 0.09–0.23 g and 16.3–20.3 cm, respectively (Table 3). Inoculation of *Paenibacillus* sp. BSR₁₋₁ immobilized in water hyacinth to soil could stimulate peanut to produce pods. However, the peanut pods have grown without *Paenibacillus* sp. BSR₁₋₁ were absent (Table 4). The reason for this is not known.

Paenibacillus sp. BSR₁₋₁ could stimulate the growth of peanut, rice, and sweet corn, and these crops also responded to the low water condition in different ways. In this study, only the peanut grown under low water conditions had a higher root dry weight when grown under full water conditions. The root dry weight of peanut grown under low water condition with *Paenibacillus* sp. BSR₁₋₁ was 0.36 g, while the root dry weight of peanut grown under full water condition with *Paenibacillus* sp. BSR₁₋₁ was only 0.25 g. In addition, the root dry weight of peanuts grown under low water conditions without *Paenibacillus* sp. BSR₁₋₁ was 0.23 g, while the root dry weight of peanut grown under full water condition without *Paenibacillus* sp. BSR₁₋₁ was only 0.09 g. However, the root dry weight of rice

and sweet corn is grown under low, and full water conditions were not significantly different. The inoculation of *Paenibacillus* sp. BSR₁₋₁ increased the root efficiency to produce shoot biomass of rice under the low water condition when considering the root-to-shoot ratio. The inoculation of *Paenibacillus* sp. BSR₁₋₁ increased the specific root length of rice under low water conditions while decreasing the specific root length of corn under both conditions. On the other hand, the inoculation of *Paenibacillus* sp. BSR₁₋₁ decreased the specific root length of peanuts under both conditions. There has been a report that high root growth under drought is usually found to increase water absorption (Farooq et al., 2009).

The number of peanut leaves planted under low water conditions was lower than those planted under full water conditions. Decreasing the leaf number is a mechanism for plants to decrease their water loss by transpiration. It is an adaptation for plants grown under drought conditions (Mohr & Schopfer, 1995). Meanwhile, the number of leaves in sweet corn planted under low and full water conditions were similar. Inoculation of *Paenibacillus* sp. BSR₁₋₁ to the soil planted with sweet corn under both conditions could increase the leaf number compared to that planted in the absence of *Paenibacillus* sp. BSR₁₋₁ (Table 3). Peanut and sweet corn responded to low water conditions in different ways. It may be due to the photosynthesis system of peanut and sweet corn, which were different. Peanuts are C3 plants, while sweet corn is a C4 plant. In general, the photorespiration rate of C3 plants is higher than in C4 plants (Mohr &

Table 3
Shoot and root growth and chlorophyll content in peanut, rice, and sweet corn leaves under full and low water conditions with and without immobilized *Paenibacillus* sp. BSR₁₋₁ inoculation

	Leaf number	Shoot length (cm)	Shoot dry weight (g)	Root length (cm)	Root dry weight (g)	Specific root length (m/g)	Root to shoot ratio	Chlorophyll a (mg/ml)	Chlorophyll b (mg/ml)	Total chlorophyll (mg/ml)
Peanut										
Low water + WH	7.0±0.6	28.42±1.2b	0.99±0.05b	20.3±1.14b	0.23±0.021b	0.87	0.23	0.38±0.03a	0.59±0.06a	0.97±0.09a
Full water + WH	10.6±0.7	44.49±2.2a	0.72±0.05c	16.3±1.54b	0.09±0.008c	1.79	0.13	0.22±0.01b	0.27±0.01b	0.49±0.01b
Low water + BSR ₁₋₁	7.7±0.3	30.72±3.4b	1.09±0.06b	28.2±2.34a	0.36±0.039a	0.79	0.33	0.34±0.02a	0.54±0.02a	0.88±0.04a
Full water + BSR ₁₋₁	10.6±0.8	39.42±2.3a	1.37±0.15a	23.4±1.65ab	0.25±0.015b	0.93	0.18	0.18±0.01b	0.31±0.04b	0.49±0.05b
Rice										
Low water + WH	5.1±0.1	26.0±2.16a	0.10±0.015b	9.0±1.22b	0.03±0.005b	3.15	0.28	13.9±2.88b	8.3±1.87b	22.2±4.74b
Full water + WH	4.7±0.1	17.6±0.99b	0.04±0.006c	8.3±0.72b	0.03±0.003b	2.79	0.65	23.5±0.09a	26.1±2.32a	49.6±2.40a
Low water + BSR ₁₋₁	5.0±0.1	29.6±0.33a	0.17±0.024a	17.4±2.79a	0.05±0.008b	3.24	0.32	13.8±0.45b	7.8±0.82b	21.5±1.26b
Full water + BSR ₁₋₁	4.8±0.1	28.8±1.28a	0.15±0.012a	16.4±1.35a	0.07±0.009a	2.42	0.45	23.4±0.11a	27.7±3.68a	51.2±3.66a
Sweet corn										
Low water + WH	3.1±0.2	24.70±2.36a	0.34±0.03b	20.29±3.25b	0.08±0.02b	2.40	0.24	10.35±0.21a	4.90±0.09a	15.24±0.12a
Full water + WH	3.4±0.2	30.82±3.43a	0.30±0.03b	20.06±2.68b	0.09±0.01b	2.36	0.28	4.50±0.01c	2.23±0.04c	6.73±0.03c
Low water + BSR ₁₋₁	4.1±0.3	27.03±1.86a	0.58±0.06a	26.75±1.81ab	0.21±0.02a	1.29	0.35	8.10±0.05b	4.18±0.33b	12.28±0.31b
Full water + BSR ₁₋₁	4.6±0.2	26.87±2.44a	0.63±0.07a	34.33±3.82a	0.17±0.02a	1.97	0.28	3.47±0.03d	1.25±0.02d	4.71±0.02d

Note. Different lower-case letters show significant differences between treatments for each plant ($P<0.05$). Abbreviations: WH = Water hyacinth; BSR₁₋₁ = *Paenibacillus* sp. BSR₁₋₁

Schopfer, 1995). Thus, C4 plants, including sweet corn in this study, can tolerate drought greater than peanut, which has a constant high photosynthetic rate.

The leaf number of rice, a C3 plant, grown under the low water condition was similar to that grown under the full condition either inoculated with or without *Paenibacillus* sp. BSR₁₋₁. The rice growth was not affected by the low water condition in this study. It may be that the rice growth was not reduced with low water conditions in this study. Rice can adapt to grow under low water conditions and can survive well. In addition, it was found that *Paenibacillus* sp. BSR₁₋₁ could promote rice growth under both low and full water conditions. The rice is grown in the presence of *Paenibacillus* sp. BSR₁₋₁ was better than that grown in the absence of *Paenibacillus* sp. BSR₁₋₁. Based on Table 3, the rice growth may be stimulated by *Paenibacillus* sp. BSR₁₋₁ with the greatest extent compared to the other plants. Inoculation of *Paenibacillus* sp. BSR₁₋₁ increased the survival of rice under both full and low water conditions; survival of rice grown under full water conditions was 77% and 34% when the soil was inoculated with and without *Paenibacillus* sp. BSR₁₋₁. Also, survival rates of 66% and 55% for rice found under low water condition inoculation with and without *Paenibacillus* sp. BSR₁₋₁ and rice are grown under the low water condition produced tillers and grain that were not observed in rice that did not receive *Paenibacillus* sp. BSR₁₋₁. *Paenibacillus* sp. BSR₁₋₁ could stimulate rice growth to the greatest extent compared to other plants because *Paenibacillus* sp.

BSR₁₋₁ was isolated from soil in a paddy field. Thus, *Paenibacillus* sp. BSR₁₋₁ may be familiar and can enhance the soil planted with rice more than soil planted with other crops. The advantage of using indigenous bacteria includes the ability to adapt to the environment after introducing the bacteria into the environment again (Kumar & Gopal, 2015). Other plant growth-promoting bacteria have been reported to stimulate rice growth under drought stress. For example, bacterial inoculation of *Bacillus* sp. EN121, EN108, and EN43 increased biomass accumulation and grain yield of *Oryza sativa* L. variety MTU1010 growth under drought stress (Joshi et al., 2020). Bacterial inoculation of *Pseudomonas jessenii* R62 and *Pseudomonas synxantha* R81 also increased growth and stress-related enzymes in *Oryza sativa* L. varieties swarna and swarna sub1 grown under drought conditions (Gusain et al., 2014).

Paenibacillus sp. BSR₁₋₁ also stimulated the growth of peanut and sweet corn. The ability of *Paenibacillus* sp. BSR₁₋₁ stimulates the growth of plants comes from its plant growth-promoting activities, including exopolysaccharide, IAA, and ammonia production. The exopolysaccharides produced by bacteria could maintain soil water and increase water and nutrient uptake of plant roots from the soil and then promote plant growth under drought conditions (Vurukonda et al., 2016). Exopolysaccharide-producing bacteria could promote plant growth under drought conditions. For example, *Planomicrobium chinense* strain P1 and *Bacillus cereus* strain P2 stimulated the growth of wheat

and promoted drought tolerance in wheat. Exopolysaccharides released from bacteria act as a rhizosheath and can protect the plant root from drought for a long time (Khan & Bano, 2019). Moreover, foliar application of exopolysaccharides from *Pantoea alhagi* NX-1 increased drought tolerance in rice seedlings. Fresh weight and relative water content in rice were increased in the presence of exopolysaccharides (Sun et al., 2020). Moreover, exopolysaccharide-producing bacteria have been reported to decrease the rice exposure to toxic ions under high salt conditions using hydroxyl and carboxyl groups in the exopolysaccharide to bind and chelate sodium ions (Shultana et al., 2020a, 2020b). This mechanism may protect plants under drought conditions, which accumulate high concentrations of ions due to the low water content in the soil.

Other roles of *Paenibacillus* sp. BSR_{1,1} stimulate the growth of plants is via IAA production. The possible important role of IAA producing bacteria in increasing plant growth under drought conditions is to modify the plant root architecture to increase the root tip number and root surface. These characteristics increase soil water and nutrient uptake (Ojuederie et al., 2019). However, an excessive amount of IAA can stimulate the transcription of genes that encode ACC synthase. This enzyme synthesizes ethylene precursors (1-aminocyclopropane-1-carboxylic acid) (Ojuederie et al., 2019). This study also revealed that each plant species responded to the IAA-producing bacteria *Paenibacillus* sp. BSR_{1,1} in different ways. The levels of

endogenous IAA response to some stress conditions within each plant tissue may be different and receiving IAA from bacteria benefits plants when the endogenous IAA is below the optimum level for plant growth (Glick, 2012). Thus, the *Paenibacillus* sp. BSR_{1,1} used in this study stimulated the growth of economic crops to different extents because each plant has different endogenous IAA levels. Moreover, the endogenous IAA level could be altered under water stress. Consequently, these crops responded to bacterial inoculation in different ways. For example, peanut growth decreased in the presence of salt stress. Inoculation of peanuts with the *Rhizobium japonicum* strain USDA-110 could alter the level of IAA in peanuts resulting in normal growth (Asim et al., 2013).

Drought usually inhibits photosynthesis in plants due to the photosynthesis pigment being destroyed by reactive oxygen species (Vurukonda et al., 2016). The lower level of chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents in leaves have been reported in several cultivated plants under drought stress, including sunflower

Table 4
The number of root nodules and pods of peanut under full and low water conditions with and without *Paenibacillus* sp. BSR_{1,1}

	Number of root nodules	Number of pods
Low water + WH	12.3±7.6	1.5±0.4
Full water + WH	none	none
Low water + BSR _{1,1}	5.6±1.6	1.8±0.2
Full water + BSR _{1,1}	2.3±0.9	1.0±0.0

Note. Abbreviations: WH = Water hyacinth; BSR_{1,1} = *Paenibacillus* sp. BSR_{1,1}

(Manivannan et al., 2007) and chickpea before flowering (Mafakheri et al., 2010). In this study, the chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents in the rice leaves grown under low water conditions were decreased in the presence or absence of *Paenibacillus* sp. BSR₁₋₁. Additionally, the low water condition did not affect the chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents in peanut and sweet corn leaves. However, the chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents in the leaves of peanut and sweet corn grew under low water conditions were higher than those grown under full water conditions (Table 3). The reason for this is not known.

The soil used in this study was acidic soil that had low organic matter (Table 5). Therefore, cropping the soil with peanut, rice, and sweet corn could increase soil fertility when considering the available phosphorus, exchangeable potassium, and

exchangeable magnesium. The amounts of available phosphorus, exchangeable potassium, and exchangeable magnesium in the soil planted with peanut, rice, and sweet corn were higher than in the unplanted soil. However, the amount of soil organic matter and the available phosphorus, exchangeable potassium, exchangeable calcium, and exchangeable magnesium in the soil inoculated with *Paenibacillus* sp. BSR₁₋₁ did not differ from the planted soil without bacterial inoculation.

CONCLUSION

Paenibacillus sp. BSR₁₋₁ immobilized with water hyacinth has the potential to stimulate the growth of economic crops under low water conditions. In addition, early flowering and fruiting were seen for peanut and rice. However, further studies with low water conditions should be conducted for agricultural purposes.

Table 5
Characteristics of soil in low water condition at the end of the experiment

	pH	Organic matter (g/kg)	% sand	% silt	% clay	Available phosphorus (mg/kg)	Exchangeable potassium (mg/kg)	Exchangeable calcium (mg/kg)	Exchangeable magnesium (mg/kg)
Soil at beginning of the experiment	4.62	6.13	48	26	26	11.0	70	929	100
Soil planted with peanut	4.72	6.48	58	22	20	21.4	82	806	114
Soil planted with peanut + <i>Paenibacillus</i> sp. BSR ₁₋₁	4.72	6.78	56	24	20	20.4	80	831	105
Soil planted with sweet corn	4.58	6.45	58	21	21	32.0	167	822	119
Soil planted with sweet corn + <i>Paenibacillus</i> sp. BSR ₁₋₁	4.54	6.15	56	22	22	21.0	182	855	123
Soil planted with rice	4.69	7.47	54	22	24	27.5	163	956	134
Soil planted with rice + <i>Paenibacillus</i> sp. BSR ₁₋₁	4.64	7.12	50	25	25	16.3	179	967	131

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