

## Maintaining Growth of Aquatic Morning Glory under Drought Condition by *Paenibacillus* sp. BSR<sub>1-1</sub>

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### Abstract

*Paenibacillus* sp. BSR<sub>1-1</sub> is a plant growth-promoting bacteria with the ability to produce indole-3-acetic acid, exopolysaccharide and ammonia. This bacterial isolate grew well under low water potential, with the concentration of polyethylene glycol (PEG) ranging from 20 - 30 % (w/v). *Paenibacillus* sp. BSR<sub>1-1</sub> was a suitable bio-inoculant to maintain the growth of aquatic morning glory cultivated under drought conditions in this study. Inoculation of *Paenibacillus* sp. BSR<sub>1-1</sub>, as immobilized cells in coconut husk, sawdust or rice straw, tended to stimulate root growth of aquatic morning glory cultivated under drought conditions. The specific root lengths of aquatic morning glory grown under drought conditions were 1.31, 2.86, and 1.76 m/g in the presence of *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized in coconut husk, sawdust or rice straw, respectively. Root length and root dry weight of aquatic morning glory planted under drought conditions in the presence of *Paenibacillus* sp. BSR<sub>1-1</sub> were around 17.71 - 18.08 cm and 0.16 - 0.28 g, respectively. Meanwhile, the root length and root dry weight of aquatic morning glory planted under drought conditions in the absence of *Paenibacillus* sp. BSR<sub>1-1</sub> were only 10.62 - 13.54 cm and 0.04 - 0.11 g, respectively. In addition, the root morphology of aquatic morning glory planted under drought conditions in the presence of *Paenibacillus* sp. BSR<sub>1-1</sub> was more branched and longer than that without *Paenibacillus* sp. BSR<sub>1-1</sub> inoculation. However, the inoculation of *Paenibacillus* sp. BSR<sub>1-1</sub> to soil did not stimulate shoot growth of aquatic morning glory planted under both normal and drought conditions.

**Keywords:** Aquatic morning glory, Drought stress, Immobilized cells, Plant growth-promoting bacteria

### Introduction

Nowadays, soil fertility in agricultural land is not sufficient to produce food for the increasing world population. Moreover, climate change is a phenomenon that decreases the fertility of the area by increasing the frequency of droughts [1,2]. Drought stress is a major agricultural obstacle that reduces crop yield in many regions of the world, including Southeast Asia, such as Thailand [3,4]. Due to climate change, the temperature in Thailand has increased by about 1.04 -1.80 °C per century and there has been a decrease in annual rainfall over the past 3 decades [3]. Drought is a serious problem and drought areas were reported in almost all parts of Thailand [5]. Drought destroys agricultural activity in several ways, including soil water deficiency, dehydration in plants, retardation of growth in plants and low agricultural productivity [6].

Many plant growth parameters decrease under drought stress, such as seed germination, seedling strength, chlorophyll content, leaf size, stem enlargement and root generation. Drought stress also decreases water-soluble nutrient diffusion to the plant roots [7,8]. Severe effects of drought results in decreasing plant productivity and plant death [9]. Various methods to resolve the drought problem in agricultural areas include using cropping systems that are less water-dependent, replacement of plant species with ones that require less water and using appropriate irrigation systems [10]. Moreover, the application of plant growth-promoting bacteria is a proposed way to alleviate drought stress in plants through improved soil quality and to promote the growth of plants under drought stress [11]. The possible mechanisms of plant growth-promoting bacteria to stimulate the growth of plants under drought stress include exopolysaccharides (EPS) production, which helps increase the soil water holding capacity [1],

indole-3-acetic acid production, which improves root growth in plants [1], 1-aminocyclopropane-1-carboxylate (ACC) deaminase production, which reduces stress from ethylene on plants and induction of the accumulation of osmolyte and antioxidant activity [8].

Using IAA-producing bacteria to alleviate drought stress and promote root growth of plants under drought stress was of interest in this study because plant roots were sensitive to drought stress and their growth was limited by drought stress before other plant organs [10]. Increasing abscisic acid and the ethylene level under drought stress usually limits the production of auxins in plants [7]. Thus, application of IAA-producing bacteria may be compensated for by endogenous auxin and to improve the growth of the plant under drought stress by modification of the root architecture (increasing the number of root tips and root surface area), which contributed to the mineral and water uptake by the plants [1,12]. IAA-producing bacteria have been reported to promote the growth and development of roots in plants grown under drought stress. For example, *Enterobacter* sp. (16i) and *Bacillus* sp. (12D6) with the abilities of IAA and salicylic acid production could change the root system architecture of wheat (*Triticum aestivum*) and maize (*Zea mays*) [12]. *Enterobacter* sp. (16i) and *Bacillus* sp. (12D6) promoted root branching in wheat and both bacteria promoted root elongation, increasing the root area and number of root tips in maize [12]. Other bacteria that promote the growth of plants under drought stress include *Azotobacter chroococcum* 67B and *Azotobacter chroococcum* 76A. Both bacteria can produce siderophores, ACC deaminase and IAA. *Azotobacter chroococcum* 67B and *Azotobacter chroococcum* 76A could decrease stress in tomatoes planted under drought stress [13].

This study focuses on using IAA-producing bacteria, *Paenibacillus* sp. BSR<sub>1-1</sub> (similarity to *Paenibacillus polymyxa* of about 97 % based on 16s rDNA sequence) to relieve drought stress in aquatic morning glory (*Ipomoea aquatic* Forsk.) cultivated under drought conditions. Aquatic morning glory is used as a model plant in this study because it is usually found in high soil moisture areas, including the edge of stagnant streams and ponds [14,15]. Thus, drought conditions may affect the growth of aquatic morning glory by decreasing their growth, resulting in dwarfism and limiting their yield production. The objective of this study was to investigate the ability of *Paenibacillus* sp. BSR<sub>1-1</sub> cells immobilized in coconut husk, sawdust and rice straw to stimulate the growth of aquatic morning glory under drought conditions. The findings of this study will be useful to improve agricultural systems under drought conditions in the future.

## Materials and methods

### Preparation of aquatic morning glory seedlings

The seeds of aquatic morning glory were obtained from Chuayongseng Seed Co. Ltd., Bangkok, Thailand. Surface sterilization of aquatic morning glory was done before planting (seeds were soaked in 0.6 % hypochlorite for 5 min and rinsed with sterilized distilled water for 1 min, 3 times). Then, 5 aquatic morning glory seeds were planting in experimental pots containing non-sterilized soil. After seed germination, 3 healthy seedlings with similar sizes were allowed to grow in the experimental pot (3 seedlings per 1 experimental pot). The other seedlings in each experimental pot were removed.

### Preparation of free cells of *Paenibacillus* sp. BSR<sub>1-1</sub>

*Paenibacillus* sp. BSR<sub>1-1</sub> was cultured in nutrient broth for 24 h at room temperature and this cell suspension was used as inoculum for the free cells. Coconut husk-, sawdust-, and rice straw-immobilized cells of *Paenibacillus* sp. BSR<sub>1-1</sub> were prepared according to the method described in Somtrakoon *et al.* [16]. These materials were cut into small pieces (5×5 cm<sup>2</sup>). Small pieces of coconut husk, sawdust and rice straw were sterilized by autoclaving at 121 °C for 15 min and were cool under room temperature. Then they were damped in the cell suspension of *Paenibacillus* sp. BSR<sub>1-1</sub> for 3 h to give immobilized cells of *Paenibacillus* sp. BSR<sub>1-1</sub> in agricultural materials. The initial number of bacterial cells from the plate count method for the coconut husk, sawdust and rice straw were 4.9, 5.0 and 6.0 log cfu/g, respectively. The plate count method was done following the method described by the U.S. Food and Drug Administration [17]

### Plant growth-promoting characteristics of *Paenibacillus* sp. BSR<sub>1-1</sub>

Preliminary screening for exopolysaccharide production was done by the method described by Lakshminarayanan [18]. Briefly, a 24 h cell suspension of *Paenibacillus* sp. BSR<sub>1-1</sub> was streaked on aniline blue agar (ABA) medium and incubated for 48 h at 30 °C. A positive test for exopolysaccharide (β-glucan) production was observed by an intense blue color colony.

NH<sub>3</sub> production was done by the method described by Ahmad *et al.* [19]. A 24 h cell suspension of *Paenibacillus* sp. BSR<sub>1-1</sub> was transferred to 10 mL of peptone water and incubated in a culture tube at 30 °C for 48 h. Then 0.5 mL Nessler's reagent was poured into the incubation tube. A positive test for NH<sub>3</sub> production was observed by the development of a brown color.

The growth of *Paenibacillus* sp. BSR<sub>1-1</sub> under low water potential was tested by the method described by Chandra *et al.* [20]. A 24 h cell suspension of *Paenibacillus* sp. BSR<sub>1-1</sub> was transferred to nutrient broth plus 20, 25 and 30 % polyethylene glycol (water potential at -0.5, -0.75 and -1.0 MPa). Nutrient broth without polyethylene glycon served as a control. Then the culture tubes were incubated for 24 h at 30 °C. Growth of the bacterial isolate was determined by optical density with a spectrophotometer at 600 nm.

### Experimental design

The experiment was performed under a factorial completely randomized design with 2 (normal condition, drought condition) × 2 factors (material with and without *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized in agricultural materials) and each treatment was performed for 7 replicates and there were 4 treatments for each material (coconut husk, sawdust or rice straw).

Coconut husk: Treatment 1 = normal condition + coconut husk, Treatment 2 = normal condition + *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized in coconut husk, Treatment 3 = drought condition + coconut husk, and Treatment 4 = drought condition + *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized in coconut husk.

Sawdust: Treatment 1 = normal condition + sawdust, Treatment 2 = normal condition + *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized in sawdust, Treatment 3 = drought condition + sawdust, and Treatment 4 = drought condition + *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized in sawdust.

Rice straw: Treatment 1 = normal condition + rice straw, Treatment 2 = normal condition + *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized in rice straw, Treatment 3 = drought condition + rice straw, and Treatment 4 = drought condition + *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized in rice straw.

The first day of the experiment was the first day of bacterial inoculation. Seven-day-old aquatic morning glory seedlings were inoculated and planted in experimental pots containing 1 kg of soil for 60 days after inoculation. Each 7 g of coconut husk, sawdust and rice straw or cells of *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized in coconut husk, sawdust and rice straw were plated on the surface of each experimental pot on the first day of the experiment. Water was spilled into the experimental pot every day to keep the normal soil moisture content at 20 mL/pot. For the drought condition, the water was added at 20 mL/pot every other day. The position of the experimental pot was changed every week to reduce the effect from the sunlight received. Plant samples were collected on day 60 of the experiment for analysis of the plant growth parameters, including shoot length, root length, shoot weight, root weight and chlorophyll contents in the leaves. The chlorophyll content in the leaves was determined according to the method described in Huang *et al.* [21].

### Statistical analysis

A two-way ANOVA and Least Square Difference (LSD) were used for variance analysis and pairwise comparison.

### Results and discussion

The results in **Table 1** show that *Paenibacillus* sp. BSR<sub>1-1</sub> is an IAA, ammonia and exopolysaccharide-producing bacteria (**Figure 1**) and it could grow at a low water potential. *Paenibacillus* sp. BSR<sub>1-1</sub> grew well at a water potential of -0.5 MPa (20 % PEG) and a decrease in the water potential to -0.75 and -1.0 MPa did not affect the growth of *Paenibacillus* sp. BSR<sub>1-1</sub>. The optical density of *Paenibacillus* sp. BSR<sub>1-1</sub> when growing at a water potential of -0.75 (25 % PEG) and -1.0 MPa (30 % PEG) was not significantly different from the optical density of *Paenibacillus* sp. BSR<sub>1-1</sub> when growing in culture media without PEG (**Table 1**).

**Table 1** Plant growth-promoting characteristics of *Paenibacillus* sp. BSR<sub>1-1</sub>.

IAA production <sup>A</sup>	Phosphate solubilization <sup>A</sup>	β-glucan production	NH <sub>3</sub> production	Drought tolerance (O.D. 600 nm) <sup>B</sup>			
				0 % PEG	20 % PEG	25 % PEG	30 % PEG
+ (ND)	- (ND)	+	+	0.45 ± 0.05b	0.73 ± 0.04a	0.48 ± 0.02b	0.42 ± 0.02b

<sup>A</sup>Data from Somtrakoon *et al.* [22] and ND mean not determined in this study

<sup>B</sup>Growth of *Paenibacillus* sp. BSR<sub>1-1</sub> in nutrient broth plus various concentrations of PEG for 24 h. Data show as mean ± SE. Values with different lowercase letters are statistically different between treatments ( $p < 0.05$ ).



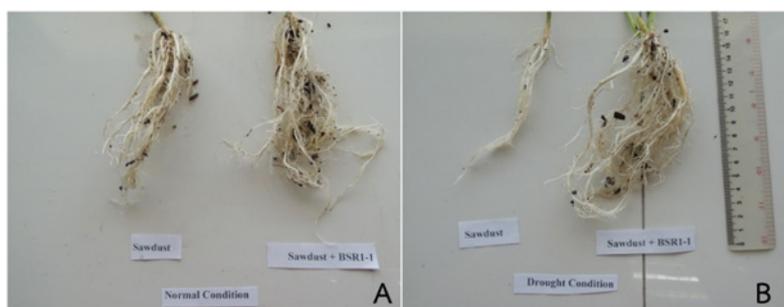
**Figure 1** Colony of *Paenibacillus* sp. BRS<sub>1-1</sub> giving blue color when grown on ABA medium for 48 h.

*Paenibacillus* sp. BRS<sub>1-1</sub> could possibly be used as a bio-inoculant to stimulate the growth of aquatic morning glory grown under drought conditions because it produces IAA, exopolysaccharides and enables growth at low water potential. In this study, *Paenibacillus* sp. BRS<sub>1-1</sub> was immobilized on 3 types of natural materials (coconut husk, sawdust and rice straw) before the addition of the bacterial cells to the soil. Immobilization of the cells was expected to protect the bacterial cells from environmental stress in soil, including suboptimal temperature, suboptimal soil moisture, protozoa predators and competition from indigenous soil microorganisms [22-26]. Thus, cells of *Paenibacillus* sp. BRS<sub>1-1</sub> immobilized on agricultural materials in this study was expected to improve the survival of the bacterial cells. The results of this study revealed that immobilized *Paenibacillus* sp. BRS<sub>1-1</sub> in coconut husk, sawdust and rice straw have similar trends to stimulate the growth of aquatic morning glory (**Figures 2 - 4**). Immobilized *Paenibacillus* sp. BRS<sub>1-1</sub> could stimulate only root growth of aquatic morning glory. The results revealed that *Paenibacillus* sp. BRS<sub>1-1</sub> immobilized on coconut husk could stimulate root growth of aquatic morning glory grown under normal and drought conditions. The results revealed that the root-shoot ratio, root length, root fresh weight and root dry weight of aquatic morning glory grown under drought conditions in the presence of chopped coconut husk immobilized *Paenibacillus* sp. BRS<sub>1-1</sub> were 0.40, 17.71 cm, 5.55 and 0.28 g, respectively. Meanwhile, the root-shoot ratio, root length, root fresh weight and root dry weight of aquatic morning glory grown under drought conditions in the absence of immobilized *Paenibacillus* sp. BRS<sub>1-1</sub> were 0.18, 13.12 cm, 1.66 and 0.08 g, respectively. The shoot fresh weight, shoot dry weight, number of shoots per plant and number of leaves per plant of aquatic morning glory grown under normal conditions or under drought conditions in the presence or absence of *Paenibacillus* sp. BRS<sub>1-1</sub> did not significantly differ.

The addition of *Paenibacillus* sp. BRS<sub>1-1</sub> either immobilized in sawdust or rice straw tended to increase the root growth of aquatic morning glory grown under drought conditions (**Figures 3 and 4**). The root length, root fresh weight and root dry weight of aquatic morning glory under drought conditions were 16.02 cm, 3.97 and 0.16 g in the presence of *Paenibacillus* sp. BRS<sub>1-1</sub> immobilized on sawdust. The root length, root fresh weight and root dry weight of aquatic morning glory under drought conditions were only 10.62 cm, 0.90 and 0.04 g in the presence of sawdust free cells of *Paenibacillus* sp. BRS<sub>1-1</sub>. Inoculation of *Paenibacillus* sp. BRS<sub>1-1</sub> immobilized on rice straw also improved the root growth of aquatic morning glory grown under drought conditions. The root length, root fresh weight and root dry weight of aquatic morning glory under drought conditions were 18.08 cm, 4.14 and 0.22 g in the presence of *Paenibacillus* sp. BRS<sub>1-1</sub> immobilized on rice straw. The root length, root fresh weight and root dry weight of *Ipomoea aquatic* under drought conditions were only 13.54 cm, 1.77 and 0.11 g in the presence of rice straw without *Paenibacillus* sp. BRS<sub>1-1</sub>. On the other hand, inoculation of *Paenibacillus* sp. BRS<sub>1-1</sub> did not improve the shoot growth of aquatic morning glory under normal conditions or drought conditions either immobilized on sawdust or rice straw.



**Figure 2** Characteristics of roots of aquatic morning glory grown under normal A) and drought conditions B) in the absence and presence of *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized in coconut husk.



**Figure 3** Characteristics of roots of aquatic morning glory grown under normal A) and drought conditions B) in the absence and presence of *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized in sawdust.



**Figure 4** Characteristics of roots of aquatic morning glory grown under normal A) and drought conditions B) in the absence and presence of *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized in rice straw.

Root growth and IAA-producing bacteria were the focus of this study because root growth is reduced first when a plant is exposed to drought stress. IAA was reported to increase plant growth under drought conditions. Exogenous IAA treatment increased the water content and chlorophyll content and improved the drought tolerance of white clover [27]. In addition, exogenous IAA could increase the water content and sugar content in wheat exposed to drought stress [28]. IAA-producing bacteria were also used to alleviate the effects of drought stress on plant growth by stimulating root proliferation and increasing water uptake [29]. In this study, *Paenibacillus* sp. BSR<sub>1-1</sub> could increase the root growth of aquatic morning glory under drought conditions. In addition, aquatic morning glory roots under drought conditions without *Paenibacillus* sp. BSR<sub>1-1</sub> inoculation seemed to be less-branched and with a lower specific root length. When *Paenibacillus* sp. BSR<sub>1-1</sub> was inoculated, the roots were more branching and the specific root length tended to increase. This was similar to that of aquatic morning glory under normal conditions. It is possible that the endogenous level of IAA, which could be disturbed by drought conditions, was modified with IAA produced by the bacterial inoculation and changed the root growth. Normally, auxin at a low concentration could induce root elongation and change the root architecture [1].

**Table 2** Shoot and root growth of aquatic morning glory inoculation with *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized in chopped coconut bract grown under normal conditions and drought conditions for 60 days. Data shown as mean ± SE.

Treatment	Shoot Growth						Root Growth			
	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Number of shoots/plant	Number of leaves/plant	Specific root length (m/g)	Root/shoot ratio	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
<b>Normal condition</b>										
Coconut	26.97 ± 1.77aB	6.44 ± 1.15aA	0.83 ± 0.16aA	2.31 ± 0.24aA	12.07 ± 1.17aA	0.44 ± 0.04aB	0.60 ± 0.03aA	16.73 ± 1.11bA	4.86 ± 0.86aA	0.47 ± 0.08aA
Coconut + BSR <sub>1-1</sub>	36.22 ± 4.53aA	5.28 ± 0.75aA	0.61 ± 0.07aA	1.69 ± 0.22aA	7.44 ± 0.52aA	0.69 ± 0.06aB	0.58 ± 0.01aA	21.79 ± 1.06aA	5.19 ± 0.67aA	0.36 ± 0.04aA
<b>Drought condition</b>										
Coconut	47.01 ± 6.48aA	6.40 ± 0.94aA	0.41 ± 0.05aA	2.12 ± 0.30aA	7.75 ± 0.92aA	1.84 ± 0.10aA	0.18 ± 0.01bB	13.12 ± 1.40bA	1.66 ± 0.34aA	0.08 ± 0.01aB
Coconut + BSR <sub>1-1</sub>	40.63 ± 2.71aA	7.31 ± 1.61aA	0.74 ± 0.22aA	1.93 ± 0.23aA	10.20 ± 1.94aA	1.31 ± 0.25aA	0.40 ± 0.04aB	17.71 ± 1.52aB	5.55 ± 1.63aA	0.28 ± 0.08aA
Condition	**	ns	ns	ns	ns	**	**	**	ns	**
Bacteria	ns	ns	ns	ns	ns	ns	**	**	ns	ns
Condition*Bacteria	ns	ns	ns	ns	ns	**	**	ns	ns	**

Values with different lowercase letters were statistically different between bacterial inoculation and non-inoculation ( $p < 0.05$ ); values with different capital letters were statistically different between the different conditions within the same inoculation type ( $p < 0.05$ ); ns, \*, \*\* denote non-significance ( $p > 0.05$ ), statistical significance ( $p < 0.05$ ) and high statistical significance ( $p < 0.01$ ) of each factor, respectively.

**Table 3** Shoot and root growth of aquatic morning glory inoculated with *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized with sawdust grown under normal conditions and drought conditions for 60 days. Data shown as mean ± SE.

Treatment	Shoot Growth						Root Growth			
	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Number of shoots/plant	Number of leaves/plant	Specific root length (m/g)	Root/shoot ratio	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
<b>Normal condition</b>										
Sawdust	26.73 ± 2.11aB	4.79 ± 0.62aA	0.76 ± 0.08aA	1.95 ± 0.19aA	8.68 ± 0.88aA	0.61 ± 0.09aB	0.90 ± 0.04aA	19.68 ± 1.15aA	6.75 ± 1.09aA	0.50 ± 0.08aA
Sawdust + BSR <sub>1-1</sub>	27.05 ± 1.74aB	5.21 ± 0.78aA	0.67 ± 0.13aA	2.05 ± 0.16aA	8.26 ± 0.66aA	0.97 ± 0.13aB	0.55 ± 0.03bA	18.38 ± 1.14aA	4.44 ± 0.93aA	0.29 ± 0.05aA
<b>Drought condition</b>										
Sawdust	52.88 ± 13.25aA	4.10 ± 1.14aA	0.38 ± 0.10aB	2.00 ± 0.32aA	7.80 ± 1.62aA	2.40 ± 0.05abA	0.12 ± 0.02aB	10.62 ± 2.23aB	0.90 ± 0.19aB	0.04 ± 0.01aB
Sawdust + BSR <sub>1-1</sub>	40.69 ± 5.50aA	5.99 ± 0.92aA	0.56 ± 0.09aA	2.08 ± 0.24aA	8.15 ± 1.12aA	2.86 ± 0.59aA	0.22 ± 0.05aB	16.02 ± 1.59aA	3.97 ± 1.00aA	0.16 ± 0.06aA
Condition	**	ns	**	ns	ns	**	**	**	**	**
Bacteria	ns	ns	ns	ns	ns	ns	**	ns	ns	ns
Condition*Bacteria	ns	ns	ns	ns	ns	ns	**	**	**	**

Values with different lowercase letters were statistically different between bacterial inoculation and non-inoculation ( $p < 0.05$ ); values with different capital letters were statistically different between the different conditions within the same inoculation type ( $p < 0.05$ ); ns, \*, \*\* denote non-significance ( $p > 0.05$ ), statistical significance ( $p < 0.05$ ) and high statistical significance ( $p < 0.01$ ) of each factor, respectively.

**Table 4** Shoot growth of aquatic morning glory inoculated with *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized with chopped rice straw grown under normal conditions and drought conditions for 60 days. Data shown as mean ± SE.

Treatment	Shoot Growth						Root Growth			
	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Number of shoots/plant	Number of leaves/plant	Specific root length (m/g)	Root/shoot ratio	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
<b>Normal condition</b>										
Straw	26.77 ± 1.72aB	6.16 ± 1.11aA	0.55 ± 0.07aA	1.82 ± 0.25aA	10.18 ± 1.02aA	0.56 ± 0.08aB	1.02 ± 0.04aA	24.35 ± 1.78aA	5.74 ± 0.84aA	0.59 ± 0.10aA
Straw + BSR <sub>1-1</sub>	32.56 ± 2.66aA	5.41 ± 0.51aA	0.49 ± 0.04aA	1.94 ± 0.21aA	9.38 ± 0.62aA	0.63 ± 0.06aB	0.70 ± 0.05bA	18.51 ± 0.88aA	7.17 ± 1.21aA	0.36 ± 0.06aA
<b>Drought condition</b>										
Straw	44.88 ± 5.87aA	6.16 ± 1.11aA	0.53 ± 0.13aA	1.82 ± 0.23aA	8.91 ± 1.38aA	1.38 ± 0.10aA	0.22 ± 0.01aB	13.54 ± 1.43aB	1.77 ± 0.42aB	0.11 ± 0.02aB
Straw + BSR <sub>1-1</sub>	37.15 ± 3.82aA	7.88 ± 1.20aA	0.68 ± 0.10aA	2.07 ± 0.23aA	8.33 ± 0.96aA	1.76 ± 0.41aA	0.27 ± 0.04aB	18.08 ± 1.19aA	4.14 ± 0.95aB	0.22 ± 0.06aA
Condition	**	ns	ns	ns	ns	**	**	**	**	**
Bacteria	ns	ns	ns	ns	ns	ns	**	ns	ns	ns
Condition*Bacteria	ns	ns	ns	ns	ns	ns	**	**	ns	*

Values with different lowercase letters were statistically different between bacterial inoculation and non-inoculation ( $p < 0.05$ ); values with different capital letters were statistically different between the different conditions within the same inoculation type ( $p < 0.05$ ); ns, \*, \*\* denote non-significance ( $p > 0.05$ ), statistical significance ( $p < 0.05$ ) and high statistical significance ( $p < 0.01$ ) of each factor, respectively.

**Table 5** Chlorophyll content in leaves of aquatic morning glory inoculated with *Paenibacillus* sp. BSR<sub>1-1</sub> immobilized with chopped coconut bract, sawdust and chopped rice straw grown under normal conditions and drought conditions for 60 days. Data shown as mean  $\pm$  SE.

Treatment	Chlorophyll a (mg/mL)	Chlorophyll b (mg/mL)	Total chlorophyll (mg/mL)	Chlorophyll a/ chlorophyll b ratio
<u>Normal condition</u>				
Coconut	17.24 $\pm$ 0.65aA	7.92 $\pm$ 0.38aA	25.15 $\pm$ 0.71aA	2.21 $\pm$ 0.14aA
Coconut + BSR <sub>1-1</sub>	17.10 $\pm$ 1.49aA	7.95 $\pm$ 0.55aA	25.04 $\pm$ 1.92aA	2.17 $\pm$ 0.14aA
<u>Drought condition</u>				
Coconut	18.14 $\pm$ 1.48aA	8.57 $\pm$ 0.59aA	26.70 $\pm$ 1.89aA	2.14 $\pm$ 0.16aA
Coconut + BSR <sub>1-1</sub>	18.98 $\pm$ 1.18aA	7.97 $\pm$ 0.76aA	26.94 $\pm$ 1.15aA	2.52 $\pm$ 0.27aA
Condition	ns	ns	ns	ns
Bacteria	ns	ns	ns	ns
Condition*Bacteria	ns	ns	ns	ns
<u>Normal condition</u>				
Sawdust	17.84 $\pm$ 1.77aA	8.30 $\pm$ 0.32aA	26.14 $\pm$ 2.02aA	2.13 $\pm$ 0.15aA
Sawdust + BSR <sub>1-1</sub>	13.51 $\pm$ 1.00aA	7.82 $\pm$ 0.53aA	21.32 $\pm$ 0.94aA	1.81 $\pm$ 0.25aA
<u>Drought condition</u>				
Sawdust	22.51 $\pm$ 3.27aA	10.46 $\pm$ 1.25aA	32.96 $\pm$ 4.46aA	2.14 $\pm$ 0.12aA
Sawdust + BSR <sub>1-1</sub>	18.19 $\pm$ 1.65aA	8.28 $\pm$ 0.83aA	26.47 $\pm$ 2.41aA	2.22 $\pm$ 0.12aA
Condition	ns	ns	ns	ns
Bacteria	ns	ns	ns	ns
Condition*Bacteria	ns	ns	ns	ns
<u>Normal condition</u>				
Straw	13.28 $\pm$ 1.32aA	6.83 $\pm$ 0.42bA	20.10 $\pm$ 1.69aB	1.92 $\pm$ 0.11aA
Straw + BSR <sub>1-1</sub>	15.99 $\pm$ 1.40aA	9.62 $\pm$ 0.86aA	25.61 $\pm$ 2.16aA	1.67 $\pm$ 0.07aA
<u>Drought condition</u>				
Straw	17.26 $\pm$ 1.53aA	8.81 $\pm$ 0.78aA	25.87 $\pm$ 2.22aA	1.94 $\pm$ 0.10aA
Straw + BSR <sub>1-1</sub>	16.74 $\pm$ 1.59aA	9.31 $\pm$ 0.74aA	26.04 $\pm$ 2.32aA	1.78 $\pm$ 0.06aA
Condition	ns	ns	ns	ns
Bacteria	ns	*	ns	ns
Condition*Bacteria	ns	ns	ns	ns

Values with different lowercase letters were statistically different between bacterial inoculation and non-inoculation ( $p < 0.05$ ); values with different capital letters were statistically different between the different conditions within the same inoculation type ( $p < 0.05$ ); ns, \*, \*\* denote non-significance ( $p > 0.05$ ), statistical significance ( $p < 0.05$ ) and high statistical significance ( $p < 0.01$ ) of each factor, respectively.

Total chlorophyll, chlorophyll a and chlorophyll b contents in aquatic morning glory leaves remained constant under normal conditions and drought conditions. Following soil inoculation with *Paenibacillus* sp. BSR<sub>1-1</sub>, the chlorophyll content in the leaves of aquatic morning glory was not significantly different when compared with non-inoculated treatments for both drought conditions and normal conditions. Normally, drought conditions reduced the photosynthesis rate, changed the photosynthetic pigment and damaged the photosynthetic apparatus. This was caused by the induction of oxidation stress and pigment degradation in the chloroplast [7,29]. The drought conditions in this study did not significantly reduce the chlorophyll content in the leaves of aquatic morning glory. This may be due to the drought conditions used in this study not representing heavy stress that could destroy chlorophyll content in aquatic morning glory leaves. Irrigation of the plant once every other day may be sufficient for maintaining the chlorophyll contents in aquatic morning glory leaves. In addition, increased, diminished or unchanged chlorophyll content in plant leaves under drought conditions have been reported in other plants [31-33]. The duration of drought and the asperity of drought were a critical factor to determine the level of chlorophyll in plants [32,33]. However, possible mechanisms of plant growth-promoting bacteria to alleviate drought stress and chlorophyll damage in plants may be due to several mechanisms, including exopolysaccharides production [1], indole-3-acetic acid production [1], ACC deaminase production and induction of the osmolyte accumulation and antioxidants activity [8].

Especially, the ability to produce exopolysaccharides by *Paenibacillus* sp. BSR<sub>1-1</sub> may be an advantage for the growth of aquatic morning glory under drought conditions. Exopolysaccharide-producing bacteria have been reported to increase the water holding capacity in soil. This characteristic of the exopolysaccharide can reduce the adverse effects of drought stress on the plant [1].

## Conclusions

Using *Paenibacillus* sp. BSR<sub>1-1</sub> as a bio-inoculant to maintain the growth of aquatic morning glory under drought conditions is possible due to its ability to produce IAA, ammonia and exopolysaccharide. Using a *Paenibacillus* sp. BSR<sub>1-1</sub> culture significantly supported the root growth of aquatic morning glory, thereby the specific root length of aquatic morning glory grown under both normal and drought conditions was usually higher than that grown without any bacterial inoculation. The use of *Paenibacillus* sp. BSR<sub>1-1</sub> to maintain the growth of other plants, as determined using field experiments, should be tested in the future.

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