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Full Length Article

Enhancing Growth of Buckwheat Sprouts and Microgreens by Endophytic Bacterium Inoculation

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Abstract

This study evaluated the effect of endophytic bacterium inoculation on sprouts and microgreens growth of common buckwheat. The endophtyic bacterium isolate ST-B2, isolated from common buckwheat seedling stems was identified to be *Herbaspirillum* sp. based on analysis of partial nucleotide sequence 16S rRNA gene. Inoculation of bacterial strain ST-B2 markedly enhanced the growth of common buckwheat sprouts and microgreens. The highest effect of seed inoculation with bacterial endophyte on sprouts growth was found in treatment of 20% (v/v) inoculum (2.0×10^{-7} cfu.mL^{-1}) while 10% (v/v) inoculum (1.0×10^{-7} cfu.mL^{-1}) had the highest effect on growth of microgreens. Inoculated sprouts growth at 25°C and 30°C showed the longer hypocotyl length and higher sprouts yield than at 20°C. Microgreens growth, fresh and dry weights increase was dependent on inoculation concentrations and days of microgreens grew. Seed inoculation and soil inoculation also exhibited the enhancing effects on microgreens growth, fresh and dry weight. The positive effects of seed and soil inoculation of *Herbaspirillum* sp. ST-B2 on sprouts and microgreens growth indicate the feasibility to increase the production yield of common buckwheat species.

Keywords: *Endophytic bacterium; Sprouts; Microgreens; Buckwheat*

Introduction

Buckwheat is not a cereal but usually grouped with cereals due to its ways of cultivation and utilization. Its two main species are common buckwheat (*Fagopyrum esculentum* Moench) and tartary buckwheat (*Fagopyrum tataricum* Gaertn.), world widely consumed, because of both seeds and sprouts are rich in nutrients, minerals and phenolic compounds (Kim *et al.*, 2001; Marton *et al.*, 2010). Several recent studies have focused on the development of buckwheat as a potential ‘‘functional food” material, particularly with respect to its seed sprouts (Kim *et al.*, 2008). Buckwheat sprouts and microgreens are a new vegetable products, which can be produced by germinated buckwheat grains for a period of times (Briatia *et al.*, 2011). Buckwheat sprouts have been introduced as a new vegetable for the first time by Kim *et al.* (2001). The sprouts of both species can be harvested at 7 or 8 days after seeding, and a greater quantity of fresh vegetable mass is commercially available (Kim *et al.* 2008). Regarding the yellow color with soft and slightly crispy texture, attractive fragrance and no bean flavor as soybean sprouts, buckwheat sprouts with removed pericarp from the cotyledons are preferred as fresh vegetables in commercial markets (Kim *et al.*, 2004). In addition, buckwheat sprouts and young seedlings (1–4 weeks old) can be consumed as a seasonal health vegetable, due to their rich content of phytonutrients, including phenolic compounds as well as rich in macro- and micro-nutrients, rutin and quercetin (Kim *et al.*, 2004; Lim *et al.*, 2012). Due to the great characteristics and high nutritional contents, the development of a new cultural methods and mass production system for buckwheat sprouts culture have been reported (Kim *et al.*, 2004; Kim *et al.*, 2001). However, buckwheat sprout cultivation techniques and growth conditions employed stills needed to improve more in order to produce a greater sprout yields.

Previous reports described the utilization of specific positive role of endophytic microorganism in stimulating plant growth and some are extensively investigated and practically utilized (Ryan *et al.*, 2008). The bacterial

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endophytes promote plant growth and the interaction of some bacterial species with hosts are extensively studied and reviewed (Rosenblueth and Martinez-Romero, 2006; Yadav *et al.*, 2010; Brader *et al.*, 2014). In buckwheat plant, previous reports are mainly on the fungal endophytes (Likar *et al.*, 2008; Zhao *et al.*, 2012), and very rare describe the use of endophytic bacteria in stimulating the growth of buckwheat (Tao *et al.*, 2004). Only one report described the positive effect of 2 bacterial strains, *Pantoae* sp. N3 and *Pantoae* sp. XJ01, on the growth and flavonoids content of tartary buckwheat (Li *et al.*, 2010). This study described the effect of newly isolated endophytic bacterium from the stem of buckwheat seedling, on growth of buckwheat sprouts and microgreens.

Materials and Methods

Buckwheat Variety and Microbial Strain

The seeds of common buckwheat variety (Suwon No. 1) were obtained from Department of Bio-Health Technology, Kangwon National University, Republic of Korea and planted at Longlanh Village, Luang Prabang, Lao PDR. The harvested seeds were used in this experiment. Endophytic bacterium, isolate ST-B2, isolated from stem of buckwheat seedling was maintained in glycerol stock at Laboratory of Sustainable Utilization of Microbial Resources, Department of Biology, Faculty of Science, Chiang Mai University. The strain was reactivated on NA plate prior using for all experiments.

Molecular Identification and Nitrogen Fixing Capability

Genomic DNA of endophytic bacterium isolate ST-B2 was isolated using method of Nishiguchi *et al.* (2002). The 16S rDNA was amplified using polymerase chain reaction (PCR) by using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3'), and the genomic DNA as the template under the following condition 94°C for 2 min; 35 cycles of 94°C for 20 s, 50°C for 20 s, and 72°C for 90 s; and a hold at 72°C for 5 min. The PCR product was purified using GF-1 96-well PCR Clean-up Kit (Vivantis, Oceanside, CA, USA) and sequenced using 3730XL DNA sequencer (Applied Biosystem, USA). Online similarity searches were performed using the BLAST algorithm of GenBank. Nitrogen fixing characteristics were investigated by observing the growth of endophytic bacterial isolate on a nitrogen-free solid agar, Burk's N-free medium, containing (per liter) 10 g glucose, 0.41 g KH_2PO_4, 0.52 g Na_2SO_4, 0.2 g $CaCl_2$, 0.1 g $MgSO_4.7H_2O$, 0.005 g $FeSO_4.7H_2O$, 0.0025 g $Na_2MoO_4.2H_2O$ and 15 g agar, pH 7±0.1 (Wilson and Knight, 1947). The bacterium was streaked on agar plate and cultivated at 37°C for 48 h. The bacterial growth was observed from the colony formed on a solid agar.

Inoculum Preparation

The endophytic bacterium isolate ST-B2 was grown in modified nutrient agar (NA). A single colony was selected and inoculated into NB medium, incubated at 37°C for 8 h under shaking conditions. Exponentially growing cells were harvested by centrifugation with 6,000 rpm at 4°C for 20 min, and re-suspended in either sterilized normal saline solutions (0.85%) to obtain the final cell densities of 10^8 cfu. mL^{-1} and used as inoculums stock for the experiment. The inoculants (treatment solutions) were prepared by mixed inoculums stock solutions with the proper dilution for the treatments using sterile distilled water to obtain a final concentration of 10% and 20% (v/v) equivalent to 1.0×10^{-7} and 2.0×10^{-7} cfu. mL^{-1}, respectively. Sterile distilled water was used as the control.

Seed Inoculation with Bacterial Endophyte and Growth of Buckwheat Sprout

Buckwheat seed was separately weighed (50 g) for each treatment and surface-sterilized with 2.5% (v/v) sodium hypochlorite for 3 min, and rinsed thoroughly in sterile distilled water for 4 times. Sterilized seeds were soaked in liquid suspension of inoculants 10% and 20% concentrations for 4 h at room temperature and the control was soaked in sterile distilled water. The treated and non-treated buckwheat seeds were transferred on the sterilized plastic net tray (27.50 cm × 15.50 cm), which was modified for sprouting buckwheat seeds. Buckwheat sprouts were cultured in a growth chamber under dark condition with temperature maintenance at 20, 25 and 30±1°C, and sterile distilled water was supplied 4 times a day. After 7 days cultivation, 10 sprouts were randomly selected from each treatment separately for investigation of root and hypocotyls length, sprout fresh weight and dry weight including moisture content.

Effect of Endophytic Bacterium on Growth of Buckwheat Microgreens

Seed inoculation: One hundred sterilized seeds were soaked in liquid suspension at concentrations of inoculants 10% and 20% (v/v), for 4 h at room temperature (25-28°C) before seeding and sterile distilled water was used for the control. The common buckwheat microgreens were grown in an open condition at temperature of 18°C/30°C (night/day). The experimental design was CRD with 3 replications. The commercial cultural soils (Natural Mix Soil 2002, OTOP, Thailand) and plastic trays (sizes 37 cm × 25 cm) were twice sterilized by autoclaving at 121°C for 30 min. The treated and non-treated seeds were sown onto the cultural trays containing 200 g sterilized soils and then again covered with 50 g of sterilized soils after seeding. Microgreens were kept grown for 7, 14 and 21 days old after germination (DAG) and distilled water was daily supplied twice a day. For morphological characteristics, ten

seedlings were randomly selected from each treatment and number of leaf per seedling, leaf sizes, root and shoot length, seedling fresh weight and dry weight and percent moisture content were investigated.

Soil inoculation: Common buckwheat seeds (100) were separately counted for each treatment and surface-sterilized with 2.5% sodium hypochlorite solutions as described previously. The cultural soils and plastic trays (37 cm × 25 cm) were twice sterilized by autoclaving at 121°C for 30 min with a 24 h interval. The inoculants were prepared with the proper dilution as described above. Common buckwheat microgreens were grown in open condition and temperature of 12°C/28°C (night/day). The experimental design was CRD, with 3 replications. The 200 g of sterilized soil was weighed into the cultural trays, and then 100 mL of liquid suspension concentrations of inoculants 10% and 20% were sprayed and thoroughly mixed, and stayed it for 30 min before seeding. Sterile distilled water was used for the control. The sterilized seeds were sown and then again covered with 50 g of sterilized soils after seeding. Thereafter, all cultural trays were maintained for seedlings growth for 7, 14 and 21 days old after germination (DAG), distilled water was daily supplied. To determine the morphological characteristics, ten seedlings were randomly selected from each treatment and the number of leaves per seedling, leaf sizes, root length, seedling height, seedling fresh weight and dry weight including percent moisture content were investigated.

Statistical Analysis

Statistical analysis of all tests was carried out using Statistix software version 8.0. FL. Data was analyzed with ANOVA and Least significant difference (LSD) test at $p \leq 0.05$ level was used to separate the means when the ANOVA F-test indicated a significant effect of the treatments.

Results

Molecular Identification and Nitrogen Fixing Characteristic of Endophytic Bacterium

The analysis of partial sequence (889 bp) of 16sRNA gene by NCBI blast algorithm indicates that the endophytic bacterium isolate ST-B2 belongs to the Genus Herbaspirillum. The nucleotide sequence of the isolate ST-B2 was 99% similar with 1445 max score to those of *Herbaspirillum* sp. ZX1, *Herbaspirillum* sp. Z19 and *Herbaspirillum rubrisubalbicans* FPs-3. It had 99% similarity with *Herbaspirillum putei* RFNB27 and *Herbaspirillum huttiense* VG AE-1 with 1339 and 1334 max score, respectively. The isolate ST-B2 was capable of growth on the Burks medium agar, a typical nitrogen free medium generally used for classification of nitrogen fixing bacteria (Fig. 1A). The diameter of *Herbaspirillum* sp. ST-B2 colony grew on N-free medium agar for 48 h was observed approximately 0.5 mm, whereas about 1.2 mm found on NA plate culture (Fig. 1B). The preliminary identification of this nitrogen fixing endophytic bacterium was concluded to be *Herbaspirillum* sp. ST-B2.

Effect of Seed Inoculation with Endophytic Bacterium on Buckwheat Sprouts Growth

Common buckwheat seed inoculation with endophytic bacterium *Herbaspirillum* sp. ST-B2 enhanced the hypocotyls and root length of common buckwheat sprouts significantly compared to the control (Table 1). The highest hypocotyl length of 19.89 cm was found in seed inoculated with 10% (v/v) concentration at 25°C growth temperature, and non-significant when compared between 10 and 20% inoculation at all growth temperatures. The highest root length was found in seed inoculated with 20% (v/v) concentration at 20°C. Comparison to the control, the non-significantly higher fresh weight per 10 sprouts was found in the sprouts grown at 20–25°C, but not for the sprout growth at 30°C. The dry weight per 10 sprouts was non-significant at all grown temperatures (Table 1). The moisture content of sprouts was 94–96% of fresh weight and showed non-significant difference compared to the control (data not shown).

In addition for the effect on total fresh weight and dry weight of sprouts, the increase was dependent on inoculation concentrations and temperature conditions. Seed inoculation with *Herbaspirillum* sp. ST-B2 significantly increased total fresh weight and dry weight of sprouts compared to the control. The highest fresh and dry weight was observed in the 20% concentrations, followed by those of 10% concentration at each temperatures compared to control (Fig. 2). In generally, sprouts hypocotyl length, whole fresh weight and dry weight were increased dependently with inoculation concentrations and temperature conditions.

Effect of Endophytic Bacterium Inoculation on Growth of Buckwheat Microgreens

Seed inoculation with endophytic bacterium *Herbaspirillum* sp. ST-B2 (10 and 20% v/v) enhanced microgreens root length and seedling height at 7, 14 and 21 after germination (DAG). It was observed that the increase in root length and seedling height of microgreens was dependent on inoculation concentrations. The number of leaf (cotyledons) per seedling was non-significant compared to control at 7 and 14 DAG. However, at 21 DAG, the number of leaf 4.7–5.3 per seedlings with increased leaf sizes (4.25×4.22 cm and 4.61×4.39 cm) at 10% and 20% (v/v) concentrations, and larger than the control (3.71×3.76 cm) (Table 2) were observed. The fresh weight and dry weight per 10 seedlings showed highest at 20% concentrations at 7 and 14 DAG, whereas at 21 DAG, increase was observed at 10% concentration and higher than of control. The moisture

Table 1: Effect of seed inoculation on growth of common buckwheat sprouts

Sprouts growth	Inoculants (% v/v)	Growth characteristics of common buckwheat sprouts			
		Hypocotyls length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
20°C	Control	15.96 ± 0.01^{b}	9.54 ± 0.09^{b}	2.47 ± 0.10^{a}	0.14 ± 0.01^{a}
	10%	16.75 ± 0.19^{a}	11.02 ± 0.82^{ab}	2.63 ± 0.18^{a}	0.15 ± 0.01^{a}
	20%	17.34 ± 0.33^{a}	12.28 ± 0.58^{a}	2.65 ± 0.01^{a}	0.17 ± 0.02^{a}
25°C	Control	16.20 ± 1.73^{b}	9.78 ± 3.93^{a}	2.81 ± 0.09^{b}	0.12 ± 0.00^{a}
	10%	19.89 ± 7.47^{a}	8.60 ± 0.71^{a}	3.47 ± 0.16^{a}	0.13 ± 0.01^{a}
	20%	18.76 ± 7.75^{a}	7.87 ± 0.13^{a}	3.09 ± 0.02^{b}	0.12 ± 0.00^{a}
30°C	Control	15.95 ± 0.35^{b}	7.77 ± 0.66^{b}	3.33 ± 0.41^{a}	0.16 ± 0.01^{a}
	10%	17.20 ± 0.57^{ab}	8.15 ± 0.49^{b}	3.11 ± 0.14^{a}	0.14 ± 0.00^{a}
	20%	17.60 ± 0.42^{a}	9.50 ± 0.42^{a}	3.05 ± 0.01^{a}	0.15 ± 0.01^{a}

Mean ± standard deviation. Values with same letter between columns differ non-significantly ($p \leq 0.05$)

Table 2: Effect of seed inoculation on growth of common buckwheat microgreens

Microgreens growth (day)	Inoculants (% v/v)	Growth characteristics of common buckwheat microgreens					
		No. of leaf	Leaf sizes (cm)	Seedling height (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
7	Control	2 ± 0.00^{a}	Nd	17.60 ± 1.46^{b}	3.59 ± 0.18^{b}	3.06 ± 0.27^{a}	0.14 ± 0.00^{a}
	10%	2 ± 0.00^{a}	Nd	19.70 ± 0.28^{ab}	4.96 ± 0.14^{a}	3.58 ± 0.44^{a}	0.15 ± 0.00^{a}
	20%	2 ± 0.00^{a}	Nd	21.00 ± 0.14^{a}	4.73 ± 0.13^{a}	3.62 ± 0.28^{a}	0.17 ± 0.03^{a}
14	Control	2 ± 0.00^{a}	Nd	20.70 ± 0.42^{b}	4.45 ± 0.66^{b}	4.02 ± 0.06^{c}	0.23 ± 0.00^{b}
	10%	2 ± 0.00^{a}	Nd	23.45 ± 1.92^{ab}	5.95 ± 0.10^{a}	4.34 ± 0.03^{b}	0.25 ± 0.01^{ab}
	20%	2.5 ± 0.28^{a}	Nd	24.90 ± 1.03^{a}	6.15 ± 0.06^{a}	5.11 ± 0.03^{a}	0.27 ± 0.01^{a}
21	Control	4.67 ± 0.4^{b}	3.71 × 3.76^{b}	28.60 ± 3.02^{a}	3.40 ± 0.19^{b}	11.56 ± 2.01^{a}	1.14 ± 0.07^{b}
	10%	5.27 ± 0.12^{a}	4.25 × 4.22^{ab}	31.10 ± 0.34^{a}	3.97 ± 0.13^{a}	14.52 ± 2.03^{a}	1.84 ± 0.19^{a}
	20%	4.87 ± 0.12^{ab}	4.61 × 4.39^{a}	30.83 ± 0.33^{a}	4.33 ± 0.37^{a}	13.47 ± 0.74^{a}	1.60 ± 0.19^{a}

Mean ± standard deviation. Values with same letter between columns differ non-significantly ($p \leq 0.05$). None detect (nd)

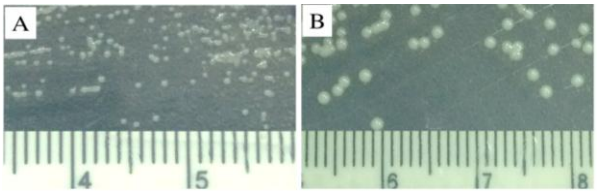

Fig. 1: Colony forming of the isolate ST-B2 after grew on nitrogen free medium at 37°C for 48 h (A) compare to the growth on nutrient agar at the same condition (B)

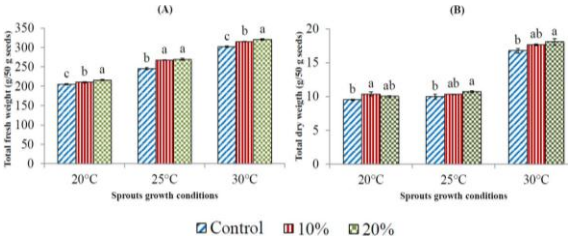

Fig. 2: Total fresh weight (A) and dry weight (B) of buckwheat sprouts from seed inoculated with *Herbaspirillum* sp. ST-B2 after grew at various temperatures for 7 days

content was 87–99% of fresh weight and showed non-significant difference compared to the control (data not shown). It was found that microgreens growth and mass production increase was dependent on the inoculation concentrations and days of microgreens grew. Total fresh weight and dry weight of seed inoculated microgreens were significant higher than the control both at 10 and 20% concentrations. Those values were 24.21 and 1.3 g at 7 DAG, 27.15 and 1.5 g at 14 DAG and 117.54 and 15 g at 21 DAG, respectively (Fig. 3A and 3C).

In case of soil inoculation, *Herbaspirillum* sp. ST-19 affected growth and increased fresh weight and dry weight of common buckwheat microgreens significantly compared to control (Table 3). Microgreens growth for 7 and 14 days after germination (DAG) showed number of leaf (2 leaves/plant) non-significantly compared to control. While, at 21 DAG, increased leaf sizes (3.79×3.84 to 4.03×3.94 cm) was larger than the control. However, soil inoculation with endophytic bacterium *Herbaspirillum* sp. at concentration of 10 and 20% (v/v) and growth for 7, 14 and 21 DAG showed increased seedling height, root length, fresh weight and dry weight. Similar to seed inoculation, the total fresh weight and dry weight were higher than of control (Fig. 3B and 3D). The moisture content was 86–94% of fresh weight and non-significantly different compared to control (data not shown).

Discussion

The endophytic bacterium *Herbaspirillum* sp. ST-B2 used in present study is quite attractive as various bacterial strains of the Genus Herbaspirillum have been reported to be effective plant growth promoting bacteria. The predominantly occurrence of the endophytic diazotrophs *Herbaspirillum* spp. in roots, stems and leaves of Gramineae

Table 3: Effect of soil inoculation on growth of common buckwheat microgreens

Microgreens growth (day)	Inoculants (% v/v)	Growth characteristics of common buckwheat microgreens					
		No. of leaf	Leaf size (cm)	Seedling height (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
7	Control	2 ± 0.00 [a]	nd	12.07 ± 0.65 [b]	3.51 ± 0.32 [b]	2.02 ± 0.22 [b]	0.12 ± 0.0 [b]
	10%	2 ± 0.00 [a]	nd	12.74 ± 0.48 [ab]	4.21 ± 0.17 [a]	2.56 ± 0.34 [a]	0.15 ± 0.01 [a]
	20%	2 ± 0.00 [a]	nd	13.45 ± 0.27 [a]	4.56 ± 0.28 [a]	2.62 ± 0.22 [a]	0.16 ± 0.01 [a]
14	Control	2 ± 0.00 [a]	nd	16.38 ± 0.40 [b]	5.19 ± 0.81 [b]	2.77 ± 0.08 [b]	0.18 ± 0.01 [a]
	10%	2 ± 0.00 [a]	nd	18.26 ± 0.93 [ab]	7.40 ± 0.03 [a]	3.56 ± 0.03 [ab]	0.21 ± 0.00 [a]
	20%	2.1 ± 0.14 [a]	nd	18.90 ± 0.48 [a]	6.41 ± 0.47 [ab]	3.86 ± 0.44 [a]	0.21 ± 0.04 [a]
21	Control	4.73 ± 0.31 [b]	3.49 × 3.40 [b]	29.28 ± 3.78 [a]	3.81 ± 0.08 [a]	12.49 ± 0.08 [a]	1.26 ± 1.44 [b]
	10%	4.93 ± 0.12 [ab]	3.79 × 3.84 [ab]	30.89 ± 1.92 [a]	4.05 ± 0.27 [a]	12.97 ± 0.27 [a]	1.68 ± 0.79 [a]
	20%	5.33 ± 0.31 [a]	4.03 × 3.94 [a]	31.82 ± 1.70 [a]	4.21 ± 0.32 [a]	13.55 ± 0.32 [a]	1.90 ± 0.32 [a]

Mean ± standard deviation. Values with same letter between columns differ non-significantly ($p \leq 0.05$). None detect (nd)

was described (Olivares *et al.*, 1997). Moreover, *Herbaspirillum rubrisubalbicans* was reported as an endophytic bacterium in sugar cane and had intracellular location in the xylem of sugar cane (Olivares *et al*., 1996). Furthermore, *Herbaspirillum seropedicae*, has been also reported in sugar cane, rice and maize, sorghum, pineaple and banana (Baldani *et al*., 1986; Weber *et al.*, 1999). On the other hand, non-plant associated *Herbaspirillum* species are also extensively described and some of them highly related to the isolate ST-B2 particularly *H. aquaticum*, *H. putei* and *H. huttiense* are found in human specimen (Regunath *et al.*, 2015). *H. putei* was found to be *H. huttiense* supsp. *huttiense* due to the high genetic relatedness (Dobritsa *et al.*, 2010). The identification in the species level of bacterial strains in this genus by 16S RNA gene sequence analysis is difficult due to their high similarity of 16S rDNA nucleotide sequence higher than 99% (Dobritsa *et al.*, 2010). However, all human pathogenic strains mentioned previously including *H. putei* and *H. huttiense* and *H. aquaticum* are reported to be negative in the growth on nitrogen free medium (Ding and Yokota, 2004), while the growth of our strain *Herbaspirillum* sp. ST-B2 was clearly positive in nitrogen free medium, Burks medium agar, a typical nitrogen free medium generally used for classification of nitrogen fixing bacteria (Wilson and Knight, 1947). Therefore, the nitrogen fixing endophytic bacterium isolate ST-B2 used in this study has high potential to be a non-pathogenic strain for human. However, more identification techniques and information are required for the complete identification at the species level.

Common buckwheat seed inoculation with endophytic bacterium *Herbaspirillum* sp. ST-B2 clearly enhanced the growth performance of the sprouts including hypocotyls and root length, total fresh and dry weight, in comparison to those of uninoculated group. However, the sprouts fresh and weight were increased dependent on inoculation concentrations and growth temperatures. This corresponds to the Li *et al.* (2010) who studied the effect of endophytic bacteria *Pantoae* sp. N3 and *Pantoae* sp. XJ01, on the growth and flavonoids content of tartary buckwheat. However, the growth promoting effect of buckwheat was also found by other microbial products, for example, treatment with exopolysaccharide (EPS) from *Bionectria*

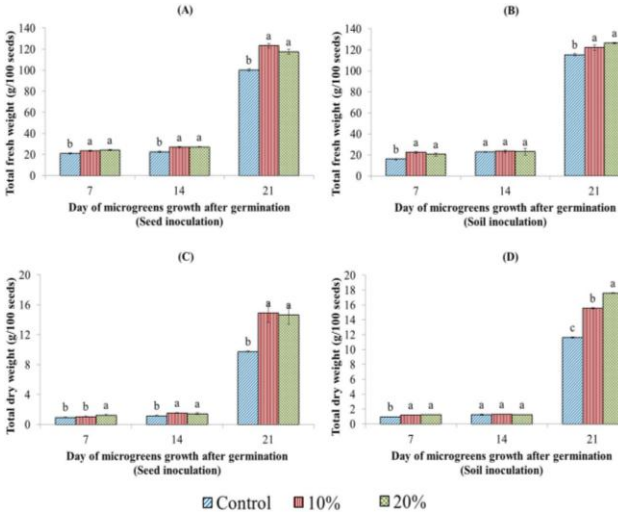

Fig. 3: Total fresh weight and dry weight of buckwheat microgreens obtained after inoculation of *Herbaspirillum* sp. ST-B2 on seed (A, C) and cultural soil (B, D) and grew at 18-30°C for 21 days

pityrodes Fat6 promoted the growth and flavonoid production in tartary buckwheat sprout cultures (Zhao *et al.*, 2015). Moreover, diterpene glycosides virescenoside A and a sum of diterpene glycoside isolated from the marine fungus *Acremonium striatisporum* MKK 4401 on buckwheat varieties Izumrud and Pri 10 showed effect on the growth of sprout root, yield and seed quality (Klykov *et al.*, 2013).

This study also clearly found that microgreens growth and mass production were increased dependent on the inoculation concentrations and days of microgreens grew both seed and soil inoculation (Fig. 3). It is markedly found that microgreens growth for 21 DAG showed more leaves and increased leaf sizes larger than control (Table 2 and 3). Recently, Yadav *et al.* (2010) reported in chickpea inoculated with plant growth promoting rhizobacteria and found that all isolates significantly increased shoot length, root length and dry matter production of chickpea seedlings. Tao *et al.* (2004) reported that some microbial inoculants applied to tartary buckwheat effectively increased young seedling growth, root length, stem width, leaf number, plant

fresh weight and dry weight, moreover, increased total flavonoids yield and soil fertility. It is suggested that soil inoculation with endophytic bacterium *Herbaspirillum* sp. ST-B2 improved microgreens growth and increased production yield as well.

Nitrogen fixing endophytic bacterium was found to be plant growth promoting regarding its function as the biofertilizer (Gupta *et al.*, 2012). The beneficial functions of endophytic bacteria on plant growth promoting were reviewed and summarized, those functions can be a consequence of nitrogen fixation or occurring by other mechanisms such as the increase of phytohormones production, biocontrol of phytopathogen in the root zone through production of antimicrobial agents or siderophore and induction of systematic acquired host resistance (Rosenblueth and Martinez-Romero, 2006). Regarding the ability of nitrogen fixing capability of the endophytic bacterium *Herbaspirillum* sp. ST-B2, growth stimulation of common buckwheat sprout are possible to be promoted by the biofertilizer, a consequence from nitrogen fixation. Seed and soil inoculation with endophytic bacterium *Herbaspirillum* sp. ST-B2 in a proper concentrations and optimum temperature condition may be recommended as a most effective for commercial sprouts and microgreens production. It is also suggested that use of endophytic bacterium as inoculants biofertilizer is an efficient approach to replace chemical fertilizers.

Conclusion

The bacterial endophyte isolate ST-B2 was classified to be *Herbaspirillum* sp.ST-B2. This study demonstrated that growing of common buckwheat sprouts, and microgreens with *Herbaspirillum* sp.ST-B2 inoculation significantly enhanced growth of sprouts and microgreens, root elongation and increase mass production of sprouts. Sprouts growth at 25 and 30°C and inoculated with 10 and 20% (v/v) concentrations respectively showed longer hypocotyls length, root elongation and increased fresh weight and dry weight of sprouts compared to the control. Similar effect on microgreens growth was observed from seed and soil inoculations with endophytic bacterium.

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References

Baldani, J.I., V.L.D. Baldani, L. Seldin and J. Döbereiner, 1986. Characterization of *Herbaspirillum seropedica*e gen. nov., sp. nov., a root-associated nitrogen-fixing bacterium. *Int. J. Syst. Evol. Microbiol.*, 36: 86–93

Brader, G., S. Compant, B. Mitter, F. Trognitz and A. Sessitsch, 2014. Metabolic potential of endophytic bacteria. *Curr. Opin. Biotechnol.*, 27: 30–37

Briatia, X., K.J. Chang, C.H. Ahn, Y.S. Lim, Y.B. Kim, S.U. Park, B.J. Park, I.J. Sung and C.H. Park, 2011. Effect of temperature, deep sea water and seed quality on growth of buckwheat sprouts. *Kor. J. Plant Res.*, 24: 724–728

Ding, L. and A. Yokota, 2004. Proposals of *Curvibacter gracilis* gen. nov., sp. nov. and *Herbaspirillum* putei sp. nov. for bacterial strains isolated from well water and reclassification of [*Pseudomonas*] *huttiensis*, [*Pseudomonas*] *lanceolata*, [*Aquaspirillum*] *delicatum* and [*Aquaspirillum*] *autotrophicum* as *Herbaspirillum huttiense* comb. nov., *Curvibacter lanceolatus* comb. nov., *Curvibacter delicatus* comb. nov. and *Herbaspirillum autotrophicum* comb. nov. *Int. J. Syst. Evol. Microbiol.*, 54: 2223–2230

Dobritsa, A.P., M.C. Reddy and M. Samadpour, 2010. Reclassification of *Herbaspirillum putei* as a later heterotypic synonym of *Herbaspirillum huttiense*, with the description of *H. huttiense* subsp. *huttiense* subsp. nov. and *H. huttiense* subsp. *putei* subsp. nov., comb. nov., and description of *Herbaspirillum aquaticum* sp. nov. *Int. J. Syst. Evol. Microbiol.*, 60: 1418–1426

Gupta, G., J. Panwar, M.S. Akhtar and P.N. Jha, 2012. Endophytic Nitrogen-fixing bacteria as biofertilizer. *In*: *Sustainable Agriculture Reviews*, pp: 183–221. Springer. Dordrecht, The Netherlands

Kim, S.J., I.S.M. Zaidul, T. Suzuki, Y. Mukasa, N. Hashimoto, S. Takigawa, T. Noda, C. Matsuura-Endo and H. Yamauchi, 2008. Comparison of phenolic compositions between common and tartary buckwheat (Fagopyrum) sprouts. *Food Chem.*, 110: 814–820

Kim, S.L., S.K. Kim and C.H. Park, 2004. Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. *Food Res. Int.*, 37: 319–327

Kim, S.L., Y.K. Son, J.J. Hwang, S.K. Kim, H.S. Hur and C.H. Park, 2001. Development and utilization of buckwheat sprouts as functional vegetables. *Fagopyrum*, 18: 49–54

Klykov, A., L. Moiseenko, E. Chaikina, S.S. Afiyatullov and M. Anisimov, 2013. Productivity and quality of seeds of *Fagopyrum esculentum* Moench under treatment with virescenosides a and a sum of glycosides of marine fungus *Acremonium striatisporum* at super low concentrations. *Russ. Agric. Sci.*, 39: 303–306

Li, X.Y., H.D. Huang, R.Y. Zhen and G.J. Niu, 2010. Identification of endophyte from tartary buckwheat and its influence on synthesis of flavonoids. *Sci. Technol. Food Ind.*, 8: 020

Likar, M., U. Bukovnik, I. Kreft, N. Chrungoo and M. Regvar, 2008. Mycorrhizal status and diversity of fungal endophytes in roots of common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*F. tataricum*). *Mycorrhiza*, 18: 309–315

Lim, J.H., K.J. Park, B.K. Kim, J.W. Jeong and H.J. Kim, 2012. Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (*Fagopyrum esculentum* M.) sprout. *Food Chem.*, 135: 1065–1070

Marton, M., Z. Mándoki, Z. Csapo-Kiss and J. Csapo, 2010. The role of sprouts in human nutrition. A review. *Acta Univ. Sapientiae*, 3: 81–117

Nishiguchi, M.K., P. Doukakis, E.M.D. Kizirian, A. Phillips, L. Prendini, H.C. Rosenbaum, E. Torres, Y. Wyner, R. DeSalle and G. Giribet, 2002. DNA isolation procedures. *In*: *Techniques in Molecular Systematics and Evolution*, pp: 247–287. DeSalle, R., G. Giribet and W.C. Wheeler (eds.). Birkhäuser Verlag. Basel, Switzerland

Olivares, F.L., V.L.D. Baldani, V.M. Reis, J.I. Baldani and J. Ddbereiner, 1996. Occurrence of the endophytic diazotrophs *Herbaspirillum* spp. in roots, stems and leaves predominantly of Gramineae. *Biol. Fertil. Soils.*, 21: 197–200

Olivares, F.L., E.K. James, J.I. Baldani and J. DÖBereiner, 1997. Infection of mottled stripe disease-susceptible and resistant sugar cane varieties by the endophytic diazotroph Herbaspirilium. *New Phytol.*, 135: 723–737

Regunath, H., J. Kimball, L.P. Smith and W. Salzer, 2015. Severe community-acquired pneumonia with bacteremia caused by *Herbaspirillum aquaticum* or *Herbaspirillum huttiense* in an immune-competent adult. *J. Clin. Microbiol.*, 53: 3086–3088

Rosenblueth, M. and E. Martinez-Romero, 2006. Bacterial endophytes and their interactions with hosts. *Mol. Plant Microbe Interact.*, 19: 827–837

Ryan, R.P., K. Germaine, A. Franks, D.J. Ryan and D.N. Dowling, 2008. Bacterial endophytes: recent developments and applications. *FEMS Microbiol. Lett.*, 278: 1–9

Tao, Y.P., Q.L. Shi, X.X. Zhang and Y.N. Zhou, 2004. Inoculation effect on growth and flavonoid content of tartary buckwheat in field experiment. *Fagopyrum*, 21: 45–50

Weber, O.B., V.L.D. Baldani, K.R.S. Teixeira, G. Kirchhof, J.I. Baldani and J. Dobereiner, 1999. Isolation and characterization of diazotrophic bacteria from banana and pineapple plants. *Plant Soil*, 210: 103–113

Wilson, P.W. and S.G. Knight, 1947. *Experiments in Bacterial Physiology*. Burgess Publishing Co, Minneapolis, Minnisota, USA

Yadav, J., J.P. Verma and K.N. Tiwari, 2010. Effect of plant growth promoting Rhizobacteria on seed germination and plant growth Chickpea (*Cicer arietinum* L.) under *in vitro* conditions. *Biol. Forum*, 2: 15–18

Zhao, G., J. Zhao, L. Peng, L. Zou, J. Wang, L. Zhong and D. Xiang, 2012. Effects of yeast polysaccharide on growth and flavonoid accumulation in *Fagopyrum tataricum* sprout cultures. *Molecules*, 17: 11335–11345

Zhao, J.L., L. Zou, L.Y. Zhong, L.X. Peng, P.L. Ying, M.L. Tan and G. Zhao, 2015. Effects of polysaccharide elicitors from endophytic *Bionectria pityrodes* Fat6 on the growth and flavonoid production in tartary buckwheat sprout cultures. *Cereal Res. Comm.*, 0: 1–11

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