Korean J. Plant Res. 29(6):712-721(2016)
https://doi.org/10.7732/kjpr.2016.29.6.712

Print ISSN 1226-3591
Online ISSN 2287-8203

Original Research Article

Effect of Endophytic Bacterium Inoculation on Seed Germination and Sprout Growth of Tartary Buckwheat

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Abstract - This experiment was conducted to investigate the endophytic bacterium *Herbaspirillum* spp effect on seed germination and sprout growth of tartary buckwheat. Inoculant concentration (%v/v) and seed soaking time were applied 10, 20 and 40% and 0, 4, 8, 12 hour, respectively. The experiment was carried out in a growth chamber maintained temperature at 20, 25 and 30ºC without light for 7 days. Results showed that, 10 to 20% (v/v) inoculant concentration by 4 to 8 h seed soaking time at 20°C temperature increased seed vigor rate and total seed germination rate 80-95% and 90-100%, respectively. On the other and, seed inoculation with *Herbaspirillum* spp. increased hypocotyl length (13-15 ㎝), root length (8-11 ㎝), total fresh weight (135-296 g) and total dry weight (7-10 g), compared to control. It is indicated that sprouts growth and yield depends on inoculation concentrations, seed soaking time and temperature. Therefore, it would be suggested that seed inoculation with *Herbaspirillum* spp. at concentration of 10 to 20% (v/v), soaking time 4 to 8 h and temperature 20°C promote seed germinations and sprout growth rate of tartary buckwheat.

Key words - Endophytic bacterium, Seed germination, Sprout growth, Tartary buckwheat

Introduction

Buckwheat is well recognized as a healthy food. Moreover, tartary buckwheat grains possess important functional food materials such as proteins, amino acid compositions, bioactive phytochemicals and mineral elements (Vojtíšková *et al*., 2012; Hsu *et al*., 2008). The major functional components have been demonstrated to be flavonoids such as rutin and quercetin (Kim *et al*., 2001; Zhao *et al*., 2012). Recently, many researchers have focused on the development of buckwheat as a potential functional food material (Lin and Zhang, 2001; Chang *et al*., 2010). Popović *et al*. (2014) reported that during the period 2010-2011 about 2.113 million ha of buckwheat was cultivated annually worldwide and the average yield was 913 kg/ha. However, the yield is still low with compared to other grain crops. Researches on buckwheat production, breeding, and cultivation have been conducted related to agricultural techniques (Akaya and Sun, 1992; Wang and Campbell, 2000).

However, seed is one of the most important inputs for higher grain production, therefore quality seed is required for rapid germination and synchronous seedling emergence, and development. Born and Corns (1958) found that a high degree of dormancy is normally exhibited by seeds of *F. esculentum* and *F. tartaricum* species at harvest and after storage in a period of time. Seed dormancy is regarded as the failure of an intact viable seed to complete germination under favorable conditions and provides an escape from suboptimal germination conditions in seasonal or spatially heterogeneous environments (Born and Corns, 1958; Wang and Campbell, 2000). Low seed vigor and seed dormancy is undesirable characteristics of farmers and researchers that results in a low productivity. Several studies on seed germination and seed emergence revealed the beneficial effects of seed priming by several ways, such as light, heat, smoke, soaking, leaching, temperature, chilling, scarification, gamma rays and salinity, including cutting or removing the seed coat and pericarp were

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reported (Koger *et al*., 2004; Mathur, 1989; Tzortzakis, 2009; Wang and Campbell, 2000). On the other hand, seed treatment with plant growth hormones such as gibberellic acid (GA_3), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), naphthalene acetic acid (NAA), and benzyladenine (BA) also reported (Dhoran and Gudadhe, 2012; Zare *et al*., 2011). However, poor seed germination is a great concern, thus, seed germination test is very important to determine the seed vigor, emergence and seedling establishment (ISTA, 1993). There are many factors influencing seed germination in general, plant growth promoting rhizobacteria, microbial inoculants have been used for treatment seed germination and seedling growth in various crops (Mia *et al*., 2012; Peng *et al*., 2009). Significant increases in seed germination and seedling vigor of agronomic important crops in response to inoculation with plant growth promoting rhizobacteria have been reported (Ertekin, 2011; Gholami *et al*., 2009; Oliveira *et al*., 2002).

With the increasing recognition of the nutritional value of buckwheat sprout, many researches on buckwheat sprout were carried out (Yoon *et al*., 2007; Zhao *et al*., 2012). The young plants (sprouts and microgreens) of buckwheat have been used as freshy health food because it takes only 6 to 8 days after sowing and could produce a greater quantity of fresh vegetables, with a soft and crispy texture and an attractive fragrance (Choi *et al*., 1996; Kim *et al*., 2004; Kim *et al*., 2008).

A high number of bacterial species have been isolated from plant tissues, such s seeds, roots, stems, and leaves (Surette *et al*., 2003). It has been demonstrated that plant stresss significantly affects endophyte communities because of plant physiological changes (Reiter *et al*., 2002). Various endophytic bacteria have been shown to have several beneficial effects on their host plant, and the mechanisms involved are probably similar to those described for rhizosphere bacteria. Plant growth promotion may be achieved through the production of plant growth enhancing substances such as indole acetic acid (Beyeler *et al*., 1999). Beneficial effects on plant growth may also be achieved by improved nutrient acquisition, including nitrogen fixation (Reiter *et al*., 2003). Endophytes may produce the enzyme 1-aminocclopropane 1-carboxylic acid (ACC) deaminase (Shah *et al*., 1998). This enzymes has no function in bacteria but cleaves ACC, the precursor of ethylene in plants and thus modulates ethylene levels which contributes to plant growth promotion (Mayak *et al*., 2004).

Nowadays, functional vegetable consumption is increasing in Asian countries due to growing awareness of health. But the production of vegetables is not enough for the requirement. Moreover, the method of production, mostly depends on chemical fertilizers and pesticides. Thus to increase the functional vegetable production without hazardous and toxic substances, the production method employed like bio-organics endophytic inoculant would be the way to ensure the quality, functional properties and safety of vegetables. Therefore, this study aims to investigate the effect of endophytic bacterium *Herbaspirillum* spp. on seed germination and sprout growth of Tartary buckwheat species.

Materials and Methods

The experiment was conducted at the Microbial Resources and Enzyme Technology Laboratory, Division of Biotechnology, Faculty of Agro-Industry, Chiang Mai University, Thailand. The seeds of tartary buckwheat variety (KW45) were obtained from a farmer of Longlanh Village, Luang Prabang, Lao PDR. Endophytic bacterium *Herbaspirillum* spp. was used for the experiment. Endophytic bacterium isolate *Herbaspirillum rubrisubalbicans* was isolated from common buckwheat seedling stems at a Laboratory of Applied Microbiology, Department of Biology, Faculty of Science, Chiang Mai University, Thailand.

Inoculums preparation

Endophytic bacterium *Herbaspirillum* spp. was cultured in a modified nutrient agar (NA) and nutrient broth (NB) (Atlas, 2010). A single colony was selected and inoculated into NB medium, following incubation at 37°C for 8-10 h under shaking conditions. Exponentially growth cells were harvested by centrifugation at 6000 rpm for 20 min, and re-suspended in either sterilized normal saline (0.85% NaCl) solutions to obtain the final cell densities of 10^8cfu/㎖ and used as inoculum stock solutions.

Seed preparation and seed inoculation

The inoculum were prepared with the proper diluents for the treatments by using sterile distilled water to obtain a final concentration of 10, 20 and 40% (v/v), respectively. Buckwheat seed was separately weighed 50 g seeds for each treatment and surface-sterilized with 2.5% (v/v) sodium hypochlorite solutions for 3 min, and rinsed thoroughly in sterile distilled water for 4 times. Seeds were soaked in liquid suspensions (10, 20 and 40%, v/v) for 0,4,8 and 12 hours at room temperature and sterile distilled water was used for the control.

Seed germination test

Tartary buckwheat seed germination tests were carried out by a top of paper (TP) method. Thirty seeds for each treatment with three replications in completely randomized design (CRD) were used. The seeds were placed directly onto a sterilized moist filter paper (No.1) in petri-dish and covered with a lid, thereafter transferred in a growth chamber under the dark condition, and temperatures maintained at 20, 25 and 30°C, for 7 days. Irrigated with sterile distilled water once in a day. Germination parameters: seed germination vigor rate (GV) in four days after seeding and total seed germination rates (GR) in seven days after seeding were investigated according to the International Rules for Seed Testing Association for the *Fagopyrum* species (ISTA, 1993), number of daily germinated seeds (GD), and seed germination index (GI) was calculated according to the formula described by Perry (1894): $GI = n/d$, where, n = number of seedlings emerging on day 'd' and d = day after planting.

Growing of tartary buckwheat sprouts

Tartary buckwheat sprouts were cultured in a growth chamber under dark condition. The treated and non-treated seeds were put on the sterilized plastic net trays (sizes 27.5 × 15.5 × 7 cm), which was modified for sprouting buckwheat seeds and kept in dark conditions for growing sprouts in a growth chamber adjusted with temperatures at 20, 25 and 30°C, for sprout growth at 7 days and water was supplied four times in a day with distilled watery sprayer (120 ㎖). Ten sprouts were randomly selected from each treatment and determined the roots and hypocotyl length, sprout fresh weight and dry weight, including the moisture content was also calculated and expressed as percent fresh weight basis.

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 and Discussion

Endophytic bacterium *Herbaspirillum* spp. inoculation effect on seed germination of tartary buckwheat

Endophytic bacterium *Herbaspirillum* spp. has positive effect on seed germination parameters such as seed germination vigor rate (GV) and total seed germination rate (GR), number of daily germinated seeds per day (GD), and seed germination index (GI), compared to control (Table 1).

In tartary buckwheat seeds, inoculation with 10 and 20% concentrations of bacterium *Herbaspirillum* with soaking for 4 and 8 hours showed higher GV (88-95%) and GR up to 100% at 20°C (Fig. 1) compared to the control. GD (6 seeds), and value of GI (14-18) were also relatively higher in 10 and 20% concentrations with soaking for 4 and 8 hours among the treatment (Table 1 & 2).

Seed germination test of tartary buckwheat at 25°C showed positive effects of bacterium *Herbaspirillum* on seed germination parameters such as GV, GR, GD, and seed GI by showing increased values significantly compared to the control (Fig. 2, Table 1 & 2). The enhancement varied depending on the treatment concentration and soaking durations. The highest germination parameters were found in the seed treated with 10 and 20% (v/v) concentrations of 4 and 8 h, which were shown GV (53-67%), GR (85-97%), GD (4 seeds), and value of GI (7-10), respectively. Seed germination test of tartary buckwheat at 30°C enhanced significantly seed germination parameters such as GV, GR, GD, and GI compared to the control. Seed inoculation with 10, 20 and 40% (v/v) concentrations and soaking for 0, 4, 8 and 12 h showed GV (55-66%), GR (62-87%), GD (3-4 seeds), and

Table 1. Effect of endophytic bacterium inoculation on number of daily germinated seeds (GD) in tartary buckwheat

Soaking time (h)	Inoculants (% v/v)	No. of daily germinated seeds		
		20°C	25°C	30°C
0	Control	5.30 ± 0.14 b[z]	2.93 ± 0.10 b	2.72 ± 0.20 a
	10	5.40 ± 0.28 a	3.36 ± 0.10 a	3.07 ± 0.10 a
	20	5.50 ± 0.14 a	3.50 ± 0.10 a	3.22 ± 0.10 a
	40	5.30 ± 0.14 b	3.36 ± 0.10 a	2.65 ± 0.30 b
4	Control	5.70 ± 0.14 a	3.22 ± 0.10 c	2.64 ± 0.10 c
	10	6.00 ± 0.00 a	3.64 ± 0.10 b	2.93 ± 0.10 b
	20	6.00 ± 0.00 a	4.00 ± 0.00 a	3.50 ± 0.10 a
	40	5.50 ± 0.14 b	3.29 ± 0.00 c	2.79 ± 0.10 bc
8	Control	6.00 ± 0.14 a	2.79 ± 0.10 c	2.79 ± 0.10 c
	10	6.00 ± 0.00 a	3.50 ± 0.10 b	3.36 ± 0.10 b
	20	6.00 ± 0.00 a	4.14 ± 0.00 a	3.71 ± 0.00 a
	40	5.50 ± 0.14 b	2.39 ± 0.10 c	2.72 ± 0.20 c
12	Control	3.30 ± 0.15 b	2.36 ± 0.10 b	2.29 ± 0.20 c
	10	3.30 ± 0.15 b	2.64 ± 0.10 a	2.93 ± 0.10 ab
	20	3.50 ± 0.05 a	3.00 ± 0.20 a	3.00 ± 0.00 a
	40	3.30 ± 0.10 b	2.64 ± 0.10 a	2.64 ± 0.10 b

[z]Means followed by a common letter between columns are non-significantly different ($p \leq 0.05$).
All the values are means (n=3) ± SD.

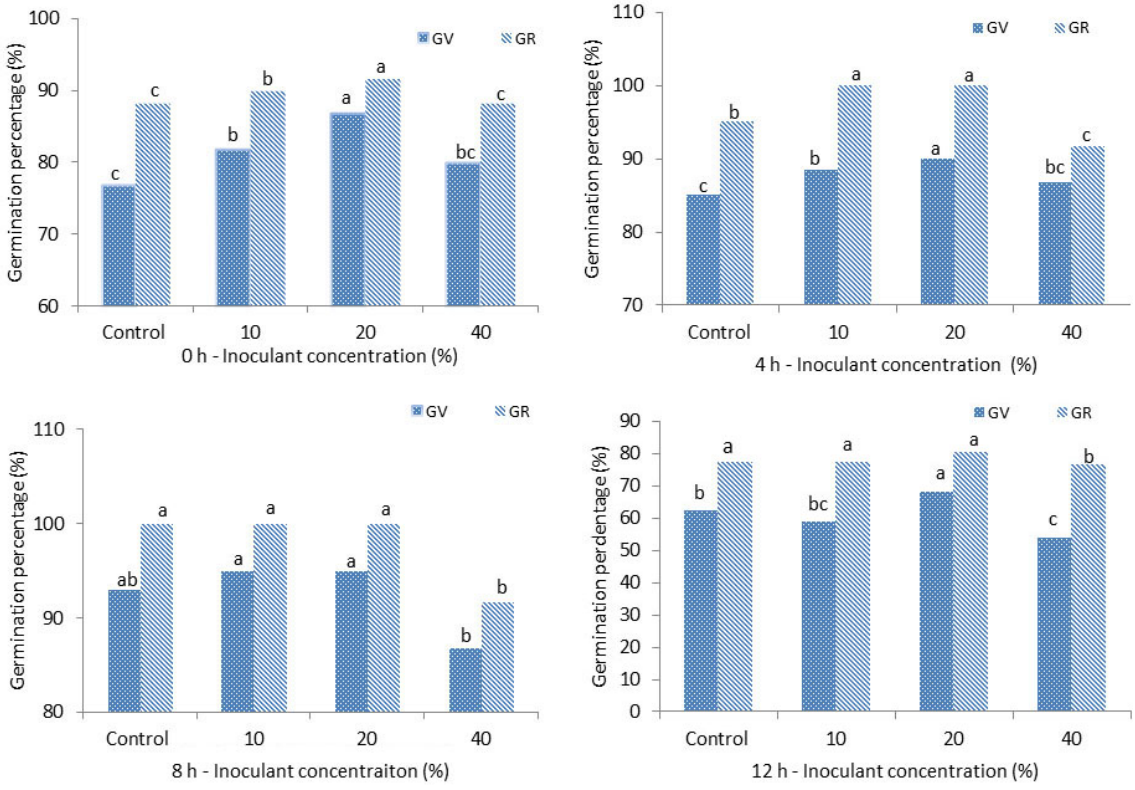

Fig. 1. Effect of endophytic bacterium inoculation on GV and GR (%) of tartary buckwheat in 0 h, 4 h, 8 h, and 12 h soaking time at 20°C. All the values are means (n=3) ± SD. Means followed by a common letter between columns are non-significantly different ($p \leq 0.05$).

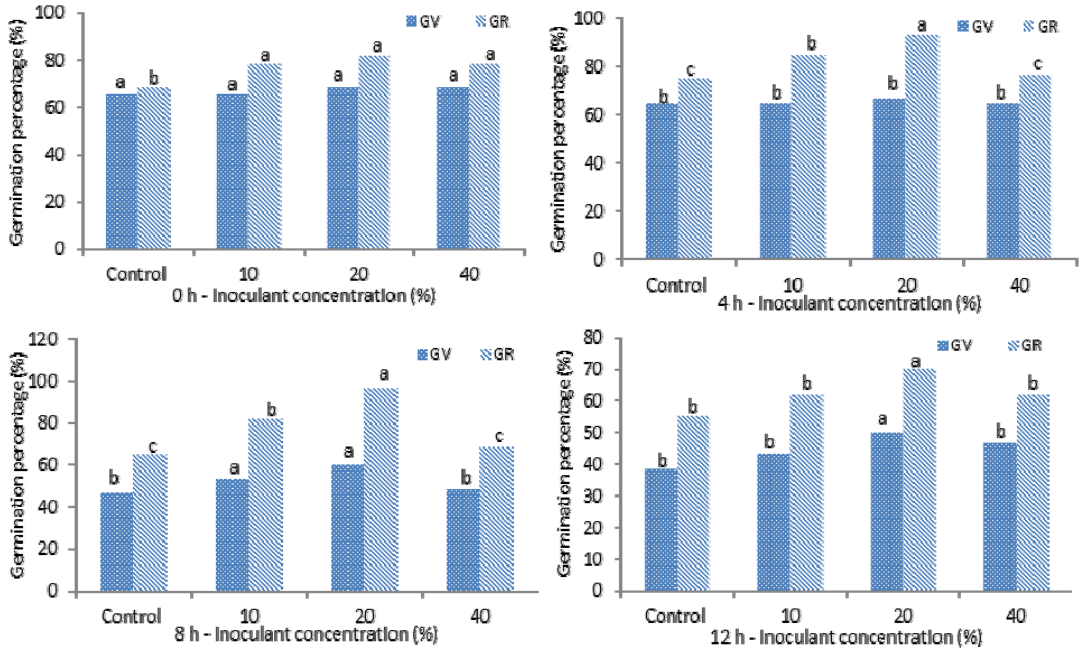

Fig. 2. Effect of endophytic bacterium inoculation on GV and GR (%) of tartary buckwheat in 0 h, 4 h, 8 h, and 12 h soaking time at 25°C . All the values are means (n=3) ± SD. Means followed by a common letter between columns are non-significantly different ($p \leq 0.05$).

Table 2. Effect of endophytic bacterium inoculation on seed germination index (GI) of tartary buckwheat

Soaking time (h)	Inoculants (% v/v)	Seed germination index		
		20°C	25°C	30°C
0	Control	16.70 ± 0.57 a[z]	11.17 ± 0.04 a	14.27 ± 0.61 a
	10	16.50 ± 1.98 a	12.95 ± 1.39 a	16.39 ± 1.15 a
	20	17.97 ± 1.08 a	12.88 ± 1.53 a	15.00 ± 0.97 a
	40	15.67 ± 3.16 b	11.93 ± 0.12 a	13.86 ± 0.71 b
4	Control	13.98 ± 1.59 b	9.80 ± 0.65 b	14.42 ± 2.24 a
	10	17.70 ± 0.05 a	10.26 ± 0.42 a	12.45 ± 0.07 a
	20	17.35 ± 1.58 a	10.21 ± 1.78 a	11.83 ± 0.39 b
	40	16.55 ± 0.02 a	9.44 ± 0.21 b	11.41 ± 0.41 b
8	Control	13.90 ± 0.65 a	6.04 ± 0.04 b	12.09 ± 0.12 a
	10	14.38 ± 0.21 a	7.31 ± 0.36 b	12.67 ± 0.19 a
	20	14.22 ± 0.45 a	9.00 ± 0.57 a	10.24 ± 0.34 b
	40	13.13 ± 0.14 b	6.81 ± 0.67 b	9.94 ± 0.33 b
12	Control	7.03 ± 0.35 b	5.06 ± 0.09 b	8.04 ± 0.06 a
	10	7.23 ± 0.32 ab	6.06 ± 1.23 a	8.89 ± 0.15 a
	20	7.94 ± 0.05 a	5.95 ± 0.15 b	8.80 ± 1.19 a
	40	6.60 ± 0.21 b	6.25 ± 0.78 a	7.89 ± 0.73 b

[z]Means followed by a common letter between columns are non-significantly different ($p \leq 0.05$).
All the values are means (n=3) ± SD.

value of GI (8-16), respectively. These might be due to the increased synthesis of hormones like gibberellins by endophytic bacterium *Herbaspirilum* spp. which would have triggered the activity of specific enzymes that promote early germination

such as α-amylase, which have brought an increase in availability of starch assimilation (Gholami *et al*., 2009). During the seed germination, α-amylase in the aleurone layer plays an important role in hydrolyzing the endosperm starch into metabolizeable sugars, which provide the energy of emergence rate and growth in cereal crops (Mia *et al*., 2012). It is indicated that endophytic bacterium *Herbaspirilum* spp. positively affect on seed germination of tartary buckwheat species. However, seed germination parameters showed slightly decreased when increased the inoculation concentrations and soaking time up to 40% and 12 h compared to other conditions. On the other hand, Mia *et al.* (2012) reported that rice seeds inoculated with rhizobium strains UMPR1006 and UMPR 1102, for 48 to 96 h improved percentage of seed emergence and lowland rice seeds inoculated with PGPR and *Rhizobium* spp. showed significantly increased seed germination rate and seedling vigor. In addition, Gholami *et al*. (2009) reported maize seeds inoculated with PGPR increased seed germination rate (90%) over non-treated seeds. Ertekin (2011) reported that *Koelreuteria paniculata* seeds treated with effective microorganism (EM1) at a concentration 100 ㎖/L and stratification for 45 days at 4°C improved seed germination rate up to 94%. On the other hand, Bharagava and Chandra (2010) reported that *Phaseolus mango* L. seed treatment with bacteria (bacteria treated post-methanated distillery effluent) at 60% concentrations and germinated at 28°C for 6 days, increased seed germination rate up to 100%, but at 20% (v/v) was found most suitable concentrations for seed germination and better plant growth than other concentrations. However, the present study found that increase inoculation concentration and soaking time up to 40% (v/v) and 12 h, decreased seed germination parameters of tartary buckwheat compared to other conditions (Table 2). Similarly, Lim *et al.* (2012) reported that decreased the seed germination rate of common buckwheat in response to increased concentrations of NaCl up to 200 mM. Dhoran and Gudadhe (2012) found in *Asparagus sprengeri* Regelin seed germination rate and vigor indexes decreased rapidly when treated with growth regulator (GA) concentration above 60 ㎎/L.

Those results were generally discussed to be attributed to the production of some phytohormones, which were enhanced seed germination. However, one of the most direct reason of the enhancements would be to seed germination and plant growth in some host plants related to the action with endophytes. This role of the endophytes in enhancing host plant performance was referred to be related to the growth hormones or secondary metabolite fungal alkaloids (Peng *et al*., 2013). Gholami *et al.* (2009) reported that maize seeds treated with PGPR strains improved seed germination rate and vigor rate over the control under *in vitro* conditions.

In contrast, increase inoculation concentrations and soaking time up to 40% and 12 h decreased seed germination parameters of tartary buckwheat compared to other conditions in this study. Bharagava and Chandra (2010) found that *Phaseolus mungo* L. seed treatment with bacteria (bacteria untreated post-methanated distillery effluent, PMDE) at high concentration (60-100%) decrease in percent seed germination to about 20-40%. On the other hand, Lim *et al.* (2012) observed a decline in the seed germination rate of common buckwheat in response to increased concentrations of NaCl up to 200 mM. Dhoran and Gudadhe (2012) found in *Asparagus sprengeri* Regelin seed germination rate and vigor indexes decreased rapidly when treated with growth regulator (GA) concentration above 60 ㎎/L. However, the present study suggested that the optimal inoculation concentration and soaking time would be in the 10 and 20% concentrations with seed soaking for 4 and 8 h. Enhancing seed germination and developing vigorous seedling are crucial for this study. In the present study, it was found that optimal temperature condition for tartary buckwheat seed germination were ranged from 20 to 30ºC, when seeds inoculated with 10 and 20% (v/v) concentrations of endophytitc bacterium *Herbaspirilum* spp. which were showed better seed germination rate. Born and Corns (1958) found that after-ripened seeds of tartary buckwheat germinated over a wide range of temperatures under laboratory conditions. Optimum seed germination and seedling emergence occurred at relatively high temperature between 20 to 30ºC for several cereal species have been reported (Koger, 2004; Gholami *et al*., 2009; Briatia *et al*., 2011, 2012; Mia *et al*., 2012).

Effect of endophytic bacterium *Herbaspirillum* spp. inoculation on sprouts growth of tartary buckwheat species

Tartary buckwheat seed inoculation with endophytic

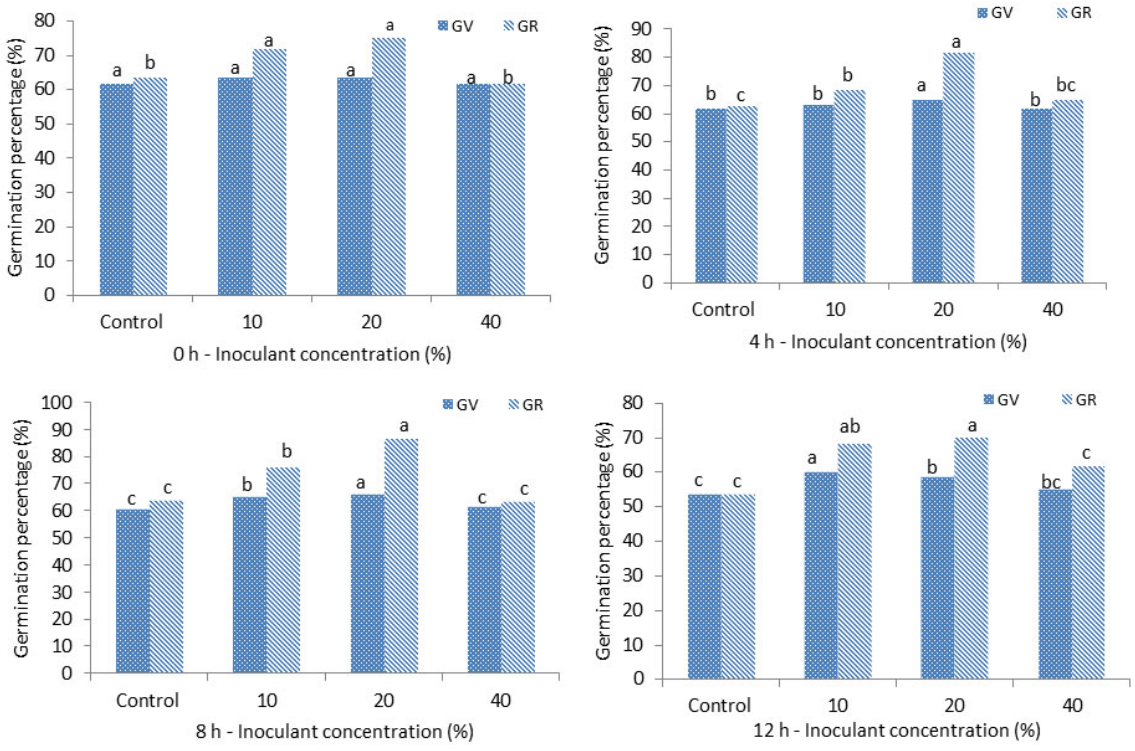

Fig. 3. Effect of endophytic bacterium inoculation on GV and GR (%) of tartary buckwheat in 0 h, 4 h, 8 h, and 12 h soaking time at 30°C . All the values are means (n=3) ± SD. Means followed by a common letter between columns are non-significantly different ($p \leq 0.05$).

Table 3. Effect of endophytic bacterium inoculation on growth of tartary buckwheat sprouts

Growing temperature (°C)	Inoculant Concent. (% v/v)	Hypocotyls length (㎝)	Root length (㎝)	Fresh weight (g/10 sprouts)	Dry weight (g/10 sprouts)
20	Control	12.70 ± 0.08 b[z]	9.76 ± 0.06 b	1.60 ± 0.10 a	0.09 ± 0.00 b
	10	13.83 ± 0.07 a	10.40 ± 0.18 ab	1.74 ± 0.08 a	0.11 ± 0.01 a
	20	14.05 ± 0.19 a	11.18 ± 0.47 a	1.81 ± 0.09 a	0.12 ± 0.01 a
	40	12.68 ± 0.42 b	9.36 ± 0.23 c	1.59 ± 0.16 a	0.09 ± 0.01 b
25	Control	14.32 ± 0.37 c	7.58 ± 0.48 b	2.10 ± 0.07 a	0.09 ± 0.00 b
	10	14.80 ± 0.25 bc	7.80 ± 0.42 b	2.10 ± 0.06 a	0.09 ± 0.00 b
	20	15.81 ± 0.10 a	8.12 ± 0.03 b	2.29 ± 0.15 a	0.12 ± 0.01 a
	40	15.23 ± 0.10 b	11.26 ± 0.76 a	2.20 ± 0.02 a	0.12 ± 0.02 a
30	Control	12.26 ± 0.28 b	6.21 ± 0.13 b	1.53 ± 0.07 a	0.06 ± 0.00 a
	10	13.09 ± 0.07 a	7.94 ± 0.11 a	1.63 ± 0.04 a	0.06 ± 0.00 a
	20	13.17 ± 0.04 a	7.62 ± 0.40 a	1.59 ± 0.11 a	0.06 ± 0.00 a
	40	12.58 ± 0.17 b	6.34 ± 0.20 b	1.57 ± 0.09 a	0.06 ± 0.00 a

[z]Means followed by a common letter between columns are non-significantly different ($p \leq 0.05$).
All the values are means (n=3) ± SD.

bacterium *Herbaspirilum* spp. enhanced hypocotyl and root length of sprouts significantly compared to control (Table 3). The highest hypocotyl and root length were found in seed treated with 20% (v/v) concentration shows 14.05 ㎝ and

11.18 ㎝ at 20ºC, 15.81 ㎝ and 8.12 ㎝ at 25ºC and 13.17 ㎝ and 7.62 ㎝ at 30°C, respectively, higher than the control (12.70 ㎝ and 9.76 ㎝ at 20°C, 14.32 ㎝ and 7.58 ㎝ at 25°C and 12.26 ㎝ and 6.21 ㎝ at 30°C). Likewise, Briatia *et al.* (2011) reported in sprouts growth at 20ºC showed longer root length than in 25 and 30ºC, whereas the hypocotyls length, fresh weight of each sprout and whole fresh weight showed highest at 30ºC. In addition, William *et al.* (1958) found that the rate of elongation of germinating seedlings was relatively constant over the temperature of 20-30ºC. On the other hand, Rahman and Saiga (2007) reported that shoot dry weight of Tall fescue (*Lolium arundinaceum* Schreb.) was significantly higher in endophyte-infected (E+) than endophyte-free (E-) plants grown in Black Andisol. However, we observed at 25ºC show better of hypocotyl length (15.81 cm) in 20% (v/v) concentration, while root length (11.26 cm) was at 40% (v/v) and higher than others (Table 3). It is indicated that endophytic bacterium function as plant growth promoting can promote plant growth by producing phytohormone, which enhanced the growth and physiological activities of the host plants. Mia *et al*. (2012) reported in rice seed inoculation with PGPR and Rhizobacteria benefited the early seedling emergence, root length, seedling growth and development. However, the fresh weight per 10 sprouts was non-significantly different compared to the control, whereas dry weight showed significantly different compared to the control and the high was in the 20% (v/v) inoculation showed 0.12 g at 20°C and 25°C, and higher than the control and others. Rahman and Saiga (2007) reported that endophyte-infected plants showed more effect on root morphology and dry matter production in tall fescue. Therefore, altered root characteristics and dry matter production could change the acquisition of nutrients when nutrient conditions vary. On the other hand, the total fresh weight of sprouts showed highest in the 10 and 20% (v/v) concentrations (139.62 and 134.50 g/50 g seeds) at 20ºC, while at 25°C showed in the 40% (215.83 g), followed by 10% (143.36 g) and 20% (141.40 g), respectively higher than those of controls. Similarly, at 30°C was also shown highest total fresh weight in the 40% (295.76 g), followed by 20% (233.94 g) and higher than the control (198.56 g). Recently, Briatia *et al*. (2012) reported that common and Tartary buckwheat seed treatment with 10% deep sea water at 25ºC slightly enhanced seed germination and sprout growth compared to non-treatment and other temperature. However, total dry weight of sprouts shown highest in the 10% (7.61 g) at 20°C, while at 25°C and 30°C showed higher in the 40% (9.71 g and 9.50 g) and higher than the control and others (Table 3). In generally, sprout hypocotyl length, whole fresh weight and dry weight were increased followed by treatment concentration and temperature conditions. Recent year, Rahman and Saiga (2007) reported that endophyte infected plants could be better competitors when soil P is limiting and the benefits of harboring endophytes would be the greatest. On the other hand, Briatia *et al*. (2011) reported that high temperature of 30ºC affect positively on the growth of buckwheat sprouts rather than low temperatures, 20 and 25ºC. Zhao *et al*. (2012) reported that growth tartary buckwheat spouts at 25ºC with yeast polysaccharide treatment resulted in high biomass and suggested a suitable time for harvesting at day 10 after seeding.

In conclusion, this study demonstrated that seed inoculation with *Herbaspirilum* spp. on tartary buckwheat in a proper concentration of 10 to 20% (v/v) and soaking time for 4 to 8 h are recommended as a most effective to promote seed germination rate, vigorous sprout production and seedling establishment in the range of the optimum temperature for seed germination and sprout growth at 20, 25, and 30ºC .

Acknowledgement

This work was supported by a grant (No. C1010713-01-01) from Kangwon National University (2014), Korea

References

Akaya, M. and J. Sun. 1992. Tendency in buckwheat research around the world. World Agriculture 92(1):25-26.

Atlas, R.M. 2010. Handbook of Microbiological Media. CRC Press Inc, Florida (USA).

Beyeler, M., C. Keel, P. Michaux and D. Haas. 1999. Enhanced production of indole-3-acetic acid by a genetically modified strain of *Pseudomonas fluorescens* CHA0 affects root growth of cucumber, but does not improve protection of the plant against Phytium root rot. FEMS Microbiol.

Ecol. 28:225-233. doi:10. 1111/j.1574-6941.1999.tb00578.

Bharagava, R.N. and R. Chandra. 2010. Effect of bacteria treated and untreated post methanated distillery effluent (PMDE) on seed germination, seedling growth and amylase activity in *Phaseolusmungo* L. Journal of Hazardous Materials 180:730-734.

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. Korean J. Plant Res. 24(6):724-728.

Briatia, X., S.K. Hong, I.J. Sung, K.J. Chang, B.J. Park and C.H. Park. 2012. Effect of deep sea water on seed germination, photoperiod and temperature on the growth and flowering of buckwheat species. Korean J. Plant Res. 25(3):323-328.

Born, W.H.V. and W.G. Corns. 1958. Studies on seed dormancy, plant development, and chemical control of tartary buckwheat (*F. tataricum* Gaertn.) I. Seed dormancy. Canadian Journal of Plant Science 38:357-365.

Chang, K.J., G.S. Seo, Y.S. Kim, D.S. Huang, J.I. Park, J.J. Park, Y.S. Lim, B.J. Park, C.H. Park and M.H. Lee. 2010. Components and biological effects of fermented extracts from tartary buckwheat sprouts. Korean J. Plant Res. 23(2):131-137.

Choi, B.H, S.L. Kim and S.K. Kim. 1996. Rutin and functional ingredients of buckwheat and their variations. Korean J. Crop Sci. 41:69-93.

Dhoran, V.S. and S.P. Gudadhe. 2012. Effect of plant growth regulators on seed germination and seedling vigour in *Asparagus sprengeri*rege lin. I. Res. J. Biological Sci. 1(7):6-10.

Ertekin, M. 2011. Effect of microorganisms, hormone treatment and stratification on seed germination of goldenrain tree (*Koelreuteria paniculata*). Int. J. Agric. Biol. 13:38-42.

Gholami, A., S. Shahsavani and S. Nezarat. 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. World Academy of Science, Engineering and Technology 25:19-24.

Hsu, C.K., B.H. Chiang, Y.S. Chen, J.H. Yang and C.L. Liu. 2008. Improving the antioxidant activity of buckwheat (*Fagopyrum tataricm* Gaertn) sprout with trace element water. Food Chemistry 108:633-641.

International Seed Testing Association (ISTA). 1993. International rules for seed testing. Seed Sci. & Technol., 21, Supplement. Switzerland.

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.

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.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 Chemistry 110:814-820.

Koger, C.H., K.N. Reddy and D.H. Poston. 2004. Factors affecting seed germination, seedling emergence, and survival of texas weed (*Caperoniapalustris*). Weed Science 52:989-995.

Lin, S.Q. and Q.H. Zhang. 2001. Advances in the development of functional foods from buckwheat. Critical Reviews in Food Science and Nutrition 41(6):451-464.

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.) sprouts. Food Chemistry 135:1065-1070.

Mathur, A. 1989. Mutation studies in buckwheat (*Fagopyrum*.) I. Effect of gamma rays on germination. Fagopyrum 9:10-14.

Mayak, S., T. Tirosh and B.R. Glick. 2004. Plant growth promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci. 166:525-530.

Mia, M.A.B., Z.H. Shamsuddin and M. Mahmood. 2012. Effect of rhizobia and plant growth promoting bacteria inoculation on germination and seedling vigor of lowland rice. African Journal of Biotechnology 11 (16):3758-3765.

Oliveira, A.L.M., S. Urquiaga, J. Dobereiner and J.I. Baldani. 2002. The effect of inoculating endophytic N_2-fixing bacteria on micropropagated sugar cane plants. Plant and Soil 242:205-215.

Peng, C.C., K.C. Chen, Y.L. Yang, L.Y. Lin and R.Y. Peng. 2009. Aqua-culture improved buckwheat sprouts with more abundant precious nutrients and hypolipidemic activity. International of Food Science and Nutrition 60(S1):232-245.

Peng, Q., C. Li, M. Song and Z. Nan. 2013. Effects of seed hydropriming on growth of *Festucasinensis* infected with *Neotyphodium* endophyte. Fungal Ecology 6:83-91.

Perry, A.D. 1984. Commentary on ISTA vigour test committee collaborative trials. Seed Sci. and Technol. 12:301-308.

Popovic, V., V. Sikora, J. Berenji, V. Filipović, Z. Dolijanović, J. Ikanović and D. Dalibor Dončić. 2014. Analysis of buckwheat production in the world and Serbia. Economics of Agriculture, EP. (61)1:53-62.

Rahman, M.H and S. Saiga 2007. Endophyte effects on nutrient acquisition in tall fescue growth in andisols. Journal of Plant Nutrient 30:2141-2158.

Reiter, B., U. Pfeifer, H. Schwab and A. Sessitsch. 2002. Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* subsp. *atroseptica.* Appl. Environ. Microbiol. 68:2261-2268. doi:10.1128/AEM.68.5.2261-2268.2002. PMID:11976096.

Reiter, B., H. Bu¨rgmann, K. Burg and A. Sessitsch. 2003. Endophytic nifH gene diversity in African sweet potato. Can. J. Microbiol. 49:549-555. doi:10.1139/w03-070. PMID:14608421.

Shah, S., J. Li, B.A. Moffatt and B.R. Glick. 1998. Isolation and characterization of ACC deaminase genes from two different plant growth-promoting rhizobacteria. Can. J. Microbiol. 44:833-843. doi:10.1139/cjm-44-9-833. PMID:9851025.

Surette, M.A., A.V. Sturz, R.R. Lada and J. Nowak. 2003. Bacterial endophytes in processing carrots (*Daucus carota L*. var. *sativus*): their localization, population density, biodiversity and their effects on plant growth. Plant Soil, 253:381-390. doi:10. 1023/A:1024835208421.

Tzortzakis, N.G. 2009. Effect of pre-sowing treatment on seed germination and seedling vigour in endive and chicory. Hort. Sci. (Prague) 36(3):117-125.

Vojtíšková, P., K. Kmentová, V. Kuban and S. Kráčmar. 2012. Chemical composition of buckwheat plant (*Fagopyrum esculentum*) and selected buckwheat products. Journal of Microbiology, Biotechnology and Food Sciences 1:1011-1019.

Wang, Y.J. and C.G. Campbell. 2000. Breaking dormancy in buckwheat. Fagopyrum 17:45-50.

William, H., V. Born and W.G. Corns. 1958. Studies on seed dormancy, plant development, and chemical control of tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.). II. Germination, growth, flowering and seed production. Canadian Journal of Plant Science 38:367-373.

Yoon, Y.H., J.G. Lee and S.K. Kim. 2007. Effect of temperature and light on growth and antioxidant contents in tartary buckwheat sprout vegetables. Proc. 7th Intl. Symp. Buckwheatat Northwest A & F University (NWAFU), Yangling, Shaanxi, China: 361-367.

Zare, A.R., M. Solouki, M. Omidi, N. Irvani, A, Oladzad Abasabadi and N. Mahdi Nezad. 2011. Effect of various treatments on seed germination and dormancy breaking in *Ferula assafoetida*L. (Asafetida), a threatened medicinal herb. Trakia Journal of Sciences 9(2):57-61.

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.

(Received 27 October 2016 ; Revised 20 October 2016 ; Accepted 23 October 2016)