Plant Pathol. J. 34(6) : 555-566 (2018)
https://doi.org/10.5423/PPJ.OA.07.2018.0130
pISSN 1598-2254 eISSN 2093-9280

Research Article Open Access

The Plant Pathology Journal
©The Korean Society of Plant Pathology

Enhanced Tolerance of Chinese Cabbage Seedlings Mediated by *Bacillus aryabhattai* H26-2 and *B. siamensis* H30-3 against High Temperature Stress and Fungal Infections

Young Hee Lee[1], Su Jeong Jang[1], Joon-Hee Han[5], Jin Su Bae[1], Hyunsuk Shin[1], Hee Jin Park[2], Mee Kyung Sang[3], Song Hee Han[4], Kyoung Su Kim[5], Sang-Wook Han[6], and Jeum Kyu Hong [1]*

[1] *Department of Horticultural Science, Gyeongnam National University of Science and Technology (GNTech), 33 Dongjin-ro, Jinju 52725, Korea*
[2] *Institute of Glocal Disease Control, Konkuk University, Seoul 05029, Korea*
[3] *National Institute of Agricultural Science, Rural Development Administration, Wanju 55365, Korea*
[4] *Hyunnong Co., Ltd, Gokseong-gun 57509, Korea*
[5] *Division of Bioresource Sciences, Kangwon National University, Chuncheon 24341, Korea*
[6] *Department of Integrative Plant Science, Chung-Ang University, Anseong 17546, Korea*

(Received on July 18, 2018; Revised on August 28, 2018; Accepted on September 10, 2018)

Two rhizobacteria *Bacillus aryabhattai* H26-2 and *B. siamensis* H30-3 were evaluated whether they are involved in stress tolerance against drought and high temperature as well as fungal infections in Chinese cabbage plants. Chinese cabbage seedlings cv. Ryeokgwang (spring cultivar) has shown better growth compared to cv. Buram-3-ho (autumn cultivar) under high temperature conditions in a greenhouse, whilst there was no difference in drought stress tolerance of the two cultivars. *In vitro* growth of *B. aryabhattai* H26-2 and *B. siamensis* H30-3 were differentially regulated under PEG 6000-induced drought stress at different growing temperatures (30, 40 and 50°C). Pretreatment with *B. aryabhattai* H26-2 and *B. siamensis* H30-3 enhanced the tolerance of Chinese cabbage seedlings to high temperature, but not to drought stress. It turns out that only *B. siamensis* H30-3 showed *in vitro* antifungal activities and *in planta* crop protection against two fungal pathogens *Alternaria brassicicola* and *Colletotrichum higginsianum* causing black spots and anthracnose on Chinese cabbage plants cv. Ryeokgwang, respectively. *B. siamensis* H30-3 brings several genes involved in production of cyclic lipopeptides in its genome and secreted hydrolytic enzymes like chitinase, protease and cellulase. *B. siamensis* H30-3 was found to produce siderophore, a high affinity iron-chelating compound. Expressions of *BrChi1* and *BrGST1* genes were up-regulated in Chinese cabbage leaves by *B. siamensis* H30-3. These findings suggest that integration of *B. aryabhattai* H26-2 and *B. siamensis* H30-3 in Chinese cabbage production system may increase productivity through improved plant growth under high temperature and crop protection against fungal pathogens.

Keywords **:** antifungal, *Bacillus*, biocontrol, Chinese cabbage, high temperature stress

Handling Associate Editor **:** Ryu, Choong-Min

Chinese cabbage production has been severely limited by drought and high temperature. Soil water deficit caused growth retardation of Chinese cabbage plants with reduced leaf area and lower fresh weight (Lee et al., 2015). Higher temperature increased the early growth of Chinese cabbage seedling but delayed the plant growth at the heading stage (Oh et al., 2014). Especially high temperature at daytime

---

*Corresponding author.
Phone) +82-55-751-3251, FAX) +82-55-751-3257
E-mail) jkhong@gntech.ac.kr
ORCID
Jeum Kyu Hong
http://orcid.org/0000-0002-9161-511X

during the reproductive growth stage resulted in increased seed number per pod but significantly declined seed weight of Chinese cabbage plants (Angadi et al., 2000). To cope with drought and high temperature stresses for a sustainable Chinese cabbage production, a few eco-friendly approaches have been suggested. Germination of Chinese cabbage seeds primed with water, KNO_3 and urea was less arrested under polyethylene glycol 6000 (PEG 6000)-mediated drought stress (Yan, 2015). Co-cultivation of Chinese cabbage plants with a root colonizing endophytic fungus *Piriformospora indica* attenuated the oxidative stress damage by PEG 4000-mediated drought (Sun et al., 2010). Since two heat-tolerant F_1 hybrids of Chinese cabbage were introduced, no appropriate cultural method was suggested for high temperature stress (Yang et al., 2001).

Rhizobacteria-mediated enhanced tolerance to environmental stresses has been suggested as one of eco-friendly strategies for crop production under harsh environmental conditions (Yang et al., 2009). Pepper plants have acquired increased tolerance during water deficit stress by *Bacillus licheniformis* K11 treatment in soils (Lim and Kim, 2013). Co-treatment with three different bacterial species *Bacillus cereus* AR156, *B. subtilis* SM21 and *Serratia* sp. XY21 induced drought tolerance in cucumber plants (Wang et al., 2012). *B. amyloliquefaciens* Bk7 and *Brevibacillus laterosporus* B4 synergistically increased survival of rice plants under dehydration stress (Kakar et al., 2016). Heat stress could be alleviated by in *Pseudomonas* sp. AKM-P6-treated sorghum plants and *Pseudomonas putida* AKMP7-treated wheat plants (Ali et al., 2009, 2011). But the beneficial microbe-mediated enhanced tolerance to high temperature stress was hardly found in plants. Plants are occasionally suffered from drought and high temperature stresses simultaneously during their growth. Physiological changes have occurred in citrus, tomato, barley and maize plants under combined drought and high temperature stress conditions (Templer et al., 2017; Zandalinas et al., 2016; Zhao et al., 2016; Zhou et al., 2017). However, it has not been investigated yet whether introducing microbes in the rhizosphere reduces loss or damage from the combined drought and high temperature stresses.

Plant diseases have been efficiently controlled by introducing rhizobacteria into the growing soils. In particular, *Bacillus* species have been demonstrated for disease suppressions in many crops and various *Bacillus*-producing lipopeptides played pivotal roles in the crop protections (Ongena and Jacques, 2008; Shafi et al., 2017). However, only a few *Bacillus*-mediated disease controls was demonstrated in Chinese cabbage plants. Chinese cabbage bottom rot caused by *Rhizoctonia solani* was reduced by *B. amuloliquefaciens* subsp. *plantarum* GR53 treatment (Kang et al., 2015). Suppressed bacterial soft rot of Chinese cabbage was mediated by *B. toyonensis* CAB12243-2 and *B. vallismortis* BS07M (Kim et al., 2018; Sang et al., 2015). Fungi *Alternaria brassicicola* and *Colletotrichum higginsianum* caused black spot and anthracnose diseases on Chinese cabbage plants, respectively. *B. amyloliquefaciens* UCM5113 and its lipopeptide extracts have *in vitro* antifungal activity against *A. brassicicola* (Asari et al., 2017). *B. subtilis* BCA31 and its culture filtrate markedly inhibited *in vitro* mycelial growth of *C. higginsianum* (Rakotoniriana et al., 2013). However, no biocontrol has been established on Chinese cabbage plants against the two fungal infections.

Although a variety of *Bacillus* spp. have exhibited their capability in soils to confer stress tolerance and disease resistance in plants, a few studies reported roles of *Bacillus aryabhattai* and *Bacillus siamensis* in enhanced plant stress tolerance. *B. aryabhattai* SRB02 improved significantly tolerance of soybean to heat stress by elevating gibberellic acid (GA) 4, GA7, GA12 and jasmonic acid levels (Park et al., 2017). *B. aryabhattai* LMA3 promoted growth of *Zea mays* under water deficit stress (Kavamura et al., 2013). Although draft genome sequence of *Bacillus siamensis* KCTC 13613^T as a plant growth-promoting bacterium was reported, any experimental evidence was not supported (Jeong et al., 2012).

In this study, roles of *B. aryabhattai* H26-2 and *B. siamensis* H30-3 in protecting Chinese cabbage plant against high temperature and/or drought stresses were investigated. *In vitro* antifungal activities and crop protection efficacies of the *B. siamensis* H30-3 species against *Alternaria brassicicola* and *Colletotrichum higginsianum* were also investigated.

Materials and Methods

Plant growth. Seeds of two Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) cultivars Ryeokgwang and Buram-3-ho were sown in plastic pots (6 cm in diameter, 5.5 cm in height) containing commercial soil mixtures (Toshil, Shinan Growth Co. Ltd., Republic of Korea). The seedlings were raised in a plastic greenhouse or in environmentally controlled walk-in growth chambers at 24 ± 2°C under a 12 h photoperiod.

Bacterial cultures and inoculation. *Bacillus aryabhattai* H26-2 and *Bacillus siamensis* H30-3 were isolated from rhizosphere of tomato plants grown in Sacheon, Gyeongnam Province. After being identified using physiological and molecular procedures, they were deposited in Korean

Agricultural Culture Collection as KACC 92165P and KACC 92187P, respectively. They were subcultured on nutrient agar (NA) media at 30°C. The two *Bacillus* species were inoculated in nutrient broth and cultured overnight by shaking at 30°C. The concentrations of bacterial suspensions in sterile water were adjusted to 4×10^7 cfu/ml using a spectrophotometer. Two-week-old Chinese cabbage seedlings in the pots were placed in the round plates (90 mm in diameter, 40 mm in height) containing 30 ml of the bacterial suspensions, and then raised for additional one week for environmental stresses as indicated in the figure legends.

For crop protection against fungal infections, a bacterial suspension (10^9 cfu/ml) of *B. siamensis* H30-3 was foliar-sprayed onto three-week-old Chinese cabbage plants (5 ml of the bacterial suspension per pot) at 1 day prior to fungal challenge inoculations.

High temperature and drought stresses on bacterial growth. *B. aryabhattai* strain H26-2 and *B. siamensis* strain H30-3 were cultured in 4 ml of nutrient broth media supplemented with different doses of PEG 6000 (0, 4, 12 and 20%) at 30, 40 and 50°C. *In vitro* growth of two phytopathogenic bacteria causing soft rot (*Pectobacterium carotovorum* subsp. *carotovorum* strain PCC21) and black rot (*Xanthomonas campestris* pv. *campestris* strain 8004) in Chinese cabbage plants were also evaluated to investigate different bacterial sensitivity under the same high temperature and/or drought stress conditions (Lee and Hong, 2012; Park et al., 2012). Bacterial inoculum density of the four bacterial suspensions was adjusted to 10^5 cfu/ml, and then optical densities at 600 nm were measured at 48 h after the liquid cultures.

High temperature and drought stresses of Chinese cabbage seedlings. Chinese cabbage seedlings were subjected to high temperature and/or drought stresses in a plastic greenhouse and environmentally controlled growth chambers.

To investigate growth of two Chinese cabbage cultivars under different ambient temperatures, seeds of two cultivars were sown in the pots at four different planting dates (4 Jul, 4 Aug, 5 Sep and 5 Oct) and grown for five weeks in the greenhouse. Thereafter, fresh weight (FW) of the seedlings was measured. Maximum and minimum temperatures in the greenhouse were measured daily, and averages in the maximum, mean and minimum temperatures for five weeks were calculated. To prevent insect infestation, the plants were grown under a mosquito net in the greenhouse.

To compare tolerance of the two Chinese cabbage cultivars to drought stress, three-week-old Chinese cabbage seedlings were undergone by two different water-stress regimes: one-week and two-weeks. After five-weeks after planting, FW of the seedlings was measured. Mock was conducted by water irrigation.

For beneficial microbe-mediated enhanced drought stress tolerance of Chinese cabbage seedlings, three-week-old Chinese cabbage seedlings pretreated with the *B. aryabhattai* H26-2 or *B. siamensis* H30-3 were grown with continuous supplement of different PEG solutions (0, 2 and 4%) under usual growing temperature conditions. FW of the seedlings was measured at 12 days after PEG treatment.

For beneficial microbe-mediated high temperature tolerance of Chinese cabbage seedlings, the seedlings were transferred to growth chambers under the controlled environments: day/night temperatures maintained at 35/25°C, 12 h light/12 h dark photoperiod, at 40% relative humidity in a growth cabinet. At five days after high temperature treatment, FW of the seedlings was measured.

For beneficial microbe-mediated tolerance to combined stresses of high temperature and drought, three-week-old Chinese cabbage seedlings pretreated with the *B. aryabhattai* H26-2 and *B. siamensis* H30-3 were cultivated with different PEG solutions (0, 2 and 4%) under high temperature conditions described above. FW of the seedlings was measured at five days after PEG-high temperature treatment.

Phytopathogenic fungal cultures. *Alternaria brassicicola* MUCL 20297 and *Colletotrichum higginsianum* C97-28 (KACC 40807) were cultured on 1/2-strength potato dextrose agar (PDA) medium at 25°C as previously described (Lee and Hong, 2014). Conidial suspension preparation and Chinese cabbage inoculation of the two fungal species were followed by the methods in our previous study (Lee and Hong, 2014).

***In vitro* dual cultures of *Bacillus* species with phytopathogenic fungi.** Antifungal activity of *B. aryabhattai* H26-2 and *B. siamensis* H30-3 was evaluated against two Chinese cabbage fungal pathogens, *A. brassicicola* MUCL 20297 and *C. higginsianum* C97-28 (KACC 40807) on 1/2-strengh PDA plates. Two *Bacillus* strains H26-2 and H30-3 were grown in nutrient broth at 30°C overnight. The bacterial suspensions of strains H26-2 and H30-3 were prepared in sterile water and adjusted to 10^8 cfu/ml. The bacterial suspension was inoculated three times on one side on PDA and mycelial plug from *A. brassicicola* and *C. higginsianum* colonies was placed on the opposite side. The plates were incubated and radii of fungal colonies were measured after 15 d and 12 d for *A. brassicicola* and *C.*

higginsianum, respectively.

Chinese cabbage inoculation and disease evaluations. Three-week-old Chinese cabbage seedlings were inoculated by foliar spraying conidial suspension (2×10^5 conidia/ml), and then placed in humid chamber for 24 h under dark condition. After the moist and dark periods, the plants were placed in growth room for symptom development. Disease was evaluated based on disease severity of 0-5 scales as follows: 0, no symptom developed; 1, several necrotic spots appeared; 2, primary true leaves blighted and necrotic spots appeared on other leaves; 3, primary and secondary true leaves blighted and necrotic spots appeared on other leaves; 4, primary and secondary true leaves blighted and 50% of other leaves collapsed; 5, whole plants completely dead.

Identification of cyclic lipopeptide biosynthetic genes in *Bacillus* spp.. *B. aryabhattai* H26-2 and *B. siamensis* H30-3 were characterised by the presence of six antimicrobial lipopeptide biosynthetic genes *bacD*, *bmyA*, *fenD*, *ituA*, *srfA* and *zwiA* involved in producing bacilysin, bacillomycin D, fengycin, iturin A, surfactin and zwittrmicin A, respectively. Single colony from *B. aryabhattai* H26-2 and *B. siamensis* H30-3 cultures on NA media was isolated and re-suspended in sterile distilled water. One µl of the bacterial suspension was subjected to a template for PCR. Primer pairs demonstrated in Kim et al. (2016) for the six antimicrobial peptide biosynthetic genes were used. PCR amplifications were carried out in 50-µl reaction mixture. PCR products were subjected to gel electrophoresis containing 1.4% agarose and RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, Republic of Korea), visualised by UV-illumination and photographed (Kim et al., 2015b).

Production of hydrolytic enzymes and siderophore from *Bacillus* spp. and phosphate solubilisation ability. Production cellulase, protease and chitinase by *B. aryabhattai* H26-2 and *B. siamensis* H30-3 were analysed on carboxymethyl cellulose agar media, Luria-Bertani agar media supplemented with 3% skim milk and colloidal chitin agar media, respectively (Han et al., 2015, 2017). Abilities of *B. aryabhattai* H26-2 and *B. siamensis* H30-3 siderophore production and phosphate solubilisation were performed on Chrome azurol S agar and Pikovskaya's agar media, respectively (Han et al., 2015, 2017).

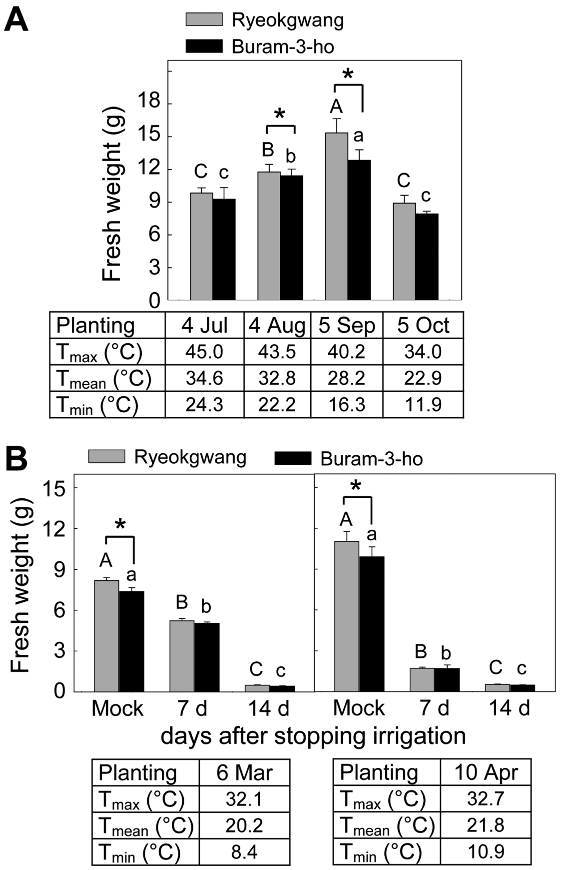
RNA isolation and defence-related gene expression analysis in Chinese cabbage leaves. Total RNA isolation and semi-quantitative RT-PCR analysis were performed with the third true leaves from three-week-old Chinese cabbage seedlings treated with or without *B. siamensis* H30-3 according to the methods of our previous studies (Kim et al., 2015b; Lee and Hong, 2014). Total RNA was isolated from the leaf tissues using RiboEx solution (GeneAll Biotechnology, Co., Ltd., Seoul, Republic of Korea), and two micrograms of the total RNA was converted to cDNA using Superscript III reverse transcriptase and oligo $(dT)_{20}$ primer according to the manufacturer's instructions (Thermo Fisher Scientific, MA, USA). PCR amplification was performed by 95°C for 30 s, 52°C for 30 s and 72°C for 30 s, with a final extension for 72°C for 10 min. RT-PCR primers which had previously demonstrated for defence-related genes of Chinese cabbage plants were used (Lee and Hong, 2012). PCR cycles of each defence-related gene were described in right parenthesis of Fig. 5C. PCR products were agarose gel electrophoresis and visualised as described above.

Statistical analyses. Error bars represent the standard errors of the means of the four independent experimental replications. Each experiment was conducted with four replications. Data were subjected to analysis of variance using SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA). Means were separated by least significant difference (LSD) test at $P < 0.05$. Graphs were prepared using SigmaPlot 10.0 (Systat Software, Inc., San Jose, CA, USA).

Results

Different plant growth of two Chinese cabbage cultivars to high temperature and drought stresses. Tolerance of two Chinese cabbage cvs. 'Ryeokgwang' and 'Buram-3-ho' to different temperature and drought stress regimes were compared (Fig. 1).

No significant difference was found in growth of five-week-old two Chinese cabbage cultivars under growing conditions (data not shown). To investigate growth of the two cultivars under high temperature condition, the two cultivars planted at the four different sowing dates (4 Jul, 4 Aug, 5 Sep and 5 Oct) were compared in a plastic house (Fig. 1A). Sowing at 5 Sep resulted in most efficient growth in both cultivars, and followed by sowing at 4 Aug. Both cultivars planted at 4 Jul and 5 Oct showed delayed the plant growth with similar levels. 'Ryeokgwang' planted at 4 Aug and 5 Sep showed better plant growth than 'Buram-3-ho' planted at the same dates. Drought tolerance of two Chinese cabbage cultivars was compared by stopping water irrigation for 7 or 14 days (Fig. 1B). At two



Planting	4 Jul	4 Aug	5 Sep	5 Oct
T_{max} (°C)	45.0	43.5	40.2	34.0
T_{mean} (°C)	34.6	32.8	28.2	22.9
T_{min} (°C)	24.3	22.2	16.3	11.9

Planting	6 Mar
T_{max} (°C)	32.1
T_{mean} (°C)	20.2
T_{min} (°C)	8.4

Planting	10 Apr
T_{max} (°C)	32.7
T_{mean} (°C)	21.8
T_{min} (°C)	10.9

Fig. 1. Different sensitivity of two Chinese cabbage seedlings (cvs. Ryeokgwang and Buram-3-ho) to high temperature and/or drought stress under greenhouse conditions. (A) Plant growth of two cultivars grown for five weeks after planting at four different planting dates (4 Jul, 4 Aug, 5 Sep and 5 Oct) under a plastic greenhouse condition. Changes in average maximum (T_{max}), average mean (T_{mean}) and average minimal (T_{min}) temperatures (°C) for five weeks under the greenhouse conditions were demonstrated. (B) Plant growth of two Chinese cabbage cultivars by stopping irrigations. Three-week-old seedlings were undergone different irrigation regimes for additional two-weeks. Error bars represent the standard errors of the means of the four independent experimental replications. Means followed by the same letter are not significantly different at 5% level by least significant difference test.

different sowing dates (6 Mar and 10 Apr), 'Ryeokgwang' grew better with higher FW than 'Buram-3-ho' with normal water irrigation (mock). However, no significant difference in plant growth of two cultivars was found at 7 or 14 days after stopping watering, although stopping watering for 14 days showed much distinctly decreased FW of two cultivars than 7 days without watering.

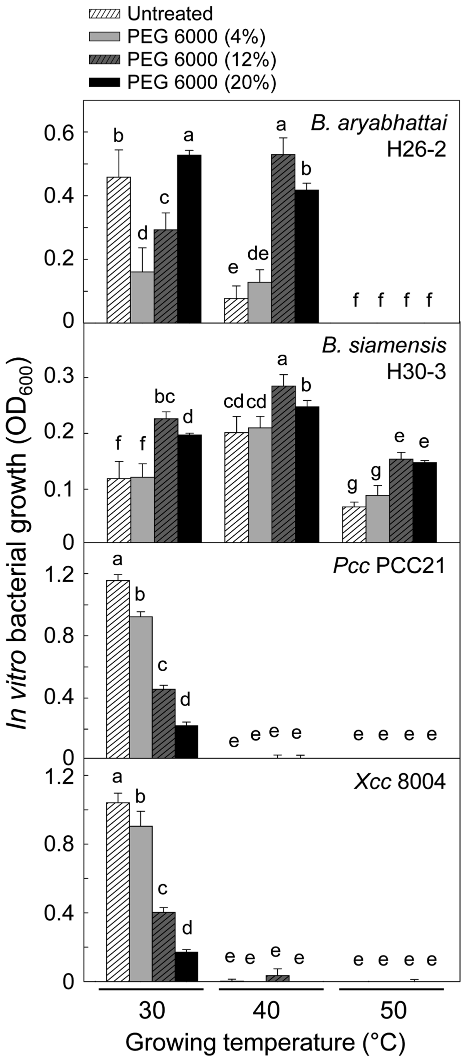


Fig. 2. Bacterial tolerance to dehydration at different growing temperatures during *in vitro* liquid cultures. Two *Bacillus* species (*B. aryabhattai* H26-2 and *B. siamensis* H30-3) and two phytopathogenic bacteria *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*) strain PCC21 and *Xanthomonas campestris* pv. *campestris* (*Xcc*) strain 8004 were cultured with increasing concentration (0, 4, 12 and 20%) of polyethylene glycol (PEG) 6000 at different temperatures (30, 40 and 50°C) for 48 h. Bacterial numbers were initially inoculated with 10^5 cfu/ml and indirectly measured using a spectrophotometer with optical density at 600 nm. Error bars represent the standard errors of the means of the four independent experimental replications. Means followed by the same letter are not significantly different at 5% level by least significant difference test.

Bacterial growth of two *Bacillus* species to drought and high temperature stresses. Tolerance of *B. aryabhattai* H26-2 and *B. siamensis* H30-3 against drought stress under different growing temperatures was evaluated (Fig. 2).

Growth of *B. aryabhattai* H26-2 was drastically suppressed by 4% PEG 6000 treatment at 30°C and increasing PEG 6000 concentration to 12% mitigated the arresting bacterial growth. Treatment with 20% PEG 6000 rather increased the bacterial growth compared to the untreated control. The higher growth temperature 40°C without PEG 6000 treatment suppressed the growth of *B. aryabhattai* H26-2 compared to its growth at 30°C. Supplement with 12% and 20% PEG 6000 enhanced the bacterial growth compared to the untreated control at 40°C. No bacterial growth of *B. aryabhattai* H26-2 occurred at 50°C with or without PEG 6000 treatment.

Relatively higher concentrations of PEG 6000 treatment (12 and 20%) accelerated *B. siamensis* H30-3 growth at each temperature (30, 40 and 50°C). The highest growing temperature at 50°C generally delayed bacterial growth compared to the other growing temperatures.

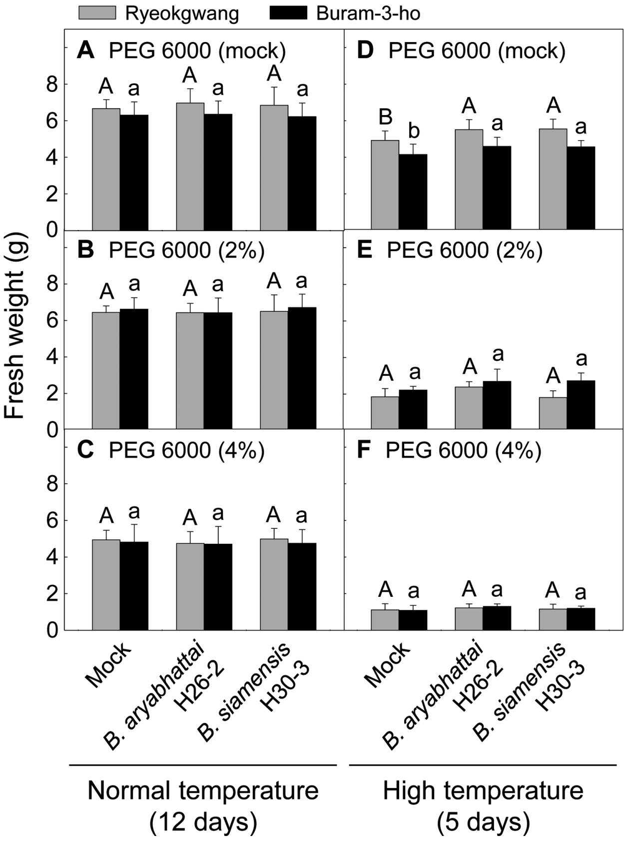


Fig. 3. Effect of pretreatment with rhizobacteria on growth of Chinese cabbage seedling (cvs. Ryeokgwang and Buram-3-ho) under drought stress at two different temperatures. Two-week-old seedlings were treated with *Bacillus aryabhattai* H26-2 and *B. siamensis* H30-3 for one week and then undergone different concentrations (0, 2 and 4%) of polyethylene glycol (PEG) 6000-mediated drought stresses under two growth temperature regimes. The seedlings were sub-irrigated with water as mocks. Fresh weight (g) of the seedlings was measured after 12 days and 5 days for normal and high temperature conditions, respectively. Error bars represent the standard errors of the means of the four independent experimental replications. Means followed by the same letter are not significantly different at 5% level by least significant difference test.

Pcc PCC21 and *Xcc* 8004 were grown well at 30°C without PEG 6000 treatment but increasing PEG 6000 resulted in suppressed bacterial growth in PEG dose-dependent manners. At 40 and 50°C, growth of *Pcc* PCC21 and *Xcc* 8004 was not detected.

High temperature and/or drought stress tolerance of two Chinese cabbage cultivars mediated by two *Bacillus* species. Tolerance of Chinese cabbage seedlings to high temperature and PEG 6000-mediated drought stresses was investigated by pretreatment with *B. aryabhattai* H26-2 and *B. siamensis* H30-3 (Fig. 3). Under normal growth temperature condition, any bacterial application did not influence plant growth with or without PEG 6000-mediated drought stress (Figs. 3A-C). Under high temperature condition without PEG 6000 treatment, i.e. under high temperature stress alone, *B. aryabhattai* H26-2 and *B. siamensis* H30-3 ameliorated the plant growth retardation in the two Chinese cabbage cultivars (Fig. 3D). However, the two *Bacillus* species did not confer any tolerance against combined stresses of high temperature and drought (Figs. 3E, F).

***In vitro* antifungal activity and *in planta* crop protection by *B. siamensis* strain H30-3.** Potent disease control activities of *B. aryabhattai* H26-2 and *B. siamensis* H30-3 were analysed in *in vitro* dual culture assays as well as in Chinese cabbage seedlings inoculated by two fungal pathogens *A. brassicicola* and *C. higginsianum* (Fig. 4).

Only *B. siamensis* H30-3 showed *in vitro* antifungal activities against two fungal species infecting Chinese cabbage plants (Figs. 4A, B). Mycelial growth of *A. brassicicola* and *C. higginsianum* was significantly limited by *B. siamensis* H30-3. That was shown as reduced sizes of the fungal colonies on PDA media at 15 d and 12 d after co-cultivation. Pretreatment of Chinese cabbage seedlings with bacterial suspension of *B. siamensis* strain H30-3 resulted in reduced disease severity against *A. brassicicola* and *C. higginsianum* (Fig. 4C). At 4 days after the fungal challenge-inoculation, disease severity of black spot and anthracnose diseases was decreased by the *B. siamensis* H30-3 in ‘ Ryeokgwang’, but not in ‘Buram-3-ho’.

Biochemical and molecular characteristics of *B. siamensis* H30-3 for crop protection. Biological roles of *B. siamensis* H30-3 were investigated to see whether or not the bacterial strain produces antifungal metabolites and/or

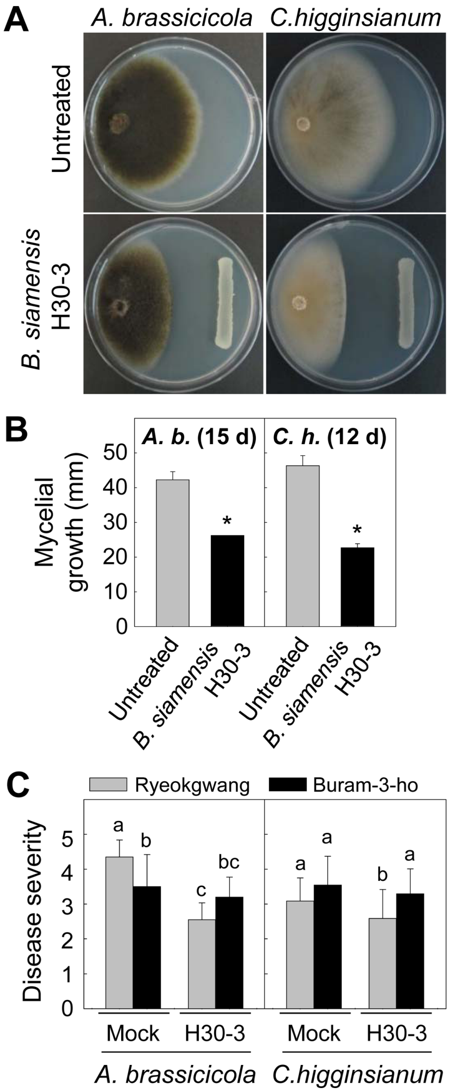


Fig. 4. Protective effects of *Bacillus siamensis* H30-3 against fungal pathogens on Chinese cabbage plants. (A) Dual culture assay for *in vitro* inhibition of mycelial growth of *Alternaria brassicicola* and *Colletotrichum higginsianum* by *B. siamensis* H30-3. The fungal pathogens were co-cultured with the bacterial strain H30-3 for 15 and 12 days at 25°C for *A. brassicicola* and *C. higginsianum,* respectively. (B) Inhibitory mycelial growth measured by half of the fungal colony diameter after co-culture. Error bars represent the standard errors of the means of the six independent experimental replications. Asterisks indicate significant differences as determined by Student's *t*-test ($P < 0.05$). (C) Reduced black spot and anthracnose disease severities on Chinese cabbage plants by the antagonistic *B. siamensis* H30-3. Bacterial suspension (10^9 cfu/ml) of *B. siamensis* H30-3 was foliar sprayed at 1 day prior to challenge inoculations of the fungal pathogens. Disease severities were evaluated at 4 days after fungal inoculation based on 0-5 scales. Error bars represent the standard errors of the means of the four independent experimental replications. Means followed by the same letter are not significantly different at 5% level by least significant difference test.

activates plant defence response (Fig. 5).

Nucleotide sequences for putative genes homologous to several genes involved in biosynthesis of cyclic lipopeptides were found in the *B. siamensis* H30-3 genome, whereas none of cyclic lipopeptide genes was detected in the *B. aryabhattai* H26-2 genome (Fig. 5A). The bacterial genes homologous to *bacD*, *bmyA*, *ituA* and *srfA* were detected in *B. siamensis* H30-3, whilst PCR products for genes homologous to *fenD* and *zwiA* were not amplified.

Several hydrolytic enzyme activities were uncovered on the media where *B. siamensis* H30-3 was grown (Fig. 5B). Strong protease and cellulase activities and relatively low chitinase activity were shown by *in vitro* culture of *B. siamensis* H30-3. Siderophore production from *B. siamensis* H30-3 was also revealed, but phosphate-solubilisation activity was not found.

Several defence-related genes in the two cultivars of Chinese cabbage leaves were differently regulated with or without *B. siamensis* H30-3 treatment (Fig. 5C). *BrChi1* and *BrGST1* expressions were up-regulated in the Chinese cabbage leaves treated with *B. siamensis* H30-3 at 1 day after foliar spraying the bacterial suspension. The induced level of *BrChi1* expression was similar in the two cultivars, but inducible *BrGST1* expression was higher in 'Ryeokgwang' than in 'Buram-3-ho'. *BrLOX2* gene expression was slightly higher in 'Ryeokgwang' than in 'Buram-3-ho' without H30-3 treatment. Pretreatment with the H30-3 did not alter the *BrLOX2* gene expression in 'Ryeokgwang', but increased the expression in 'Buram-3-ho'. Expression of *BrBGL2* and *BrAPX1* genes was not altered by the bacterial treatment.

Discussion

Global concerns are increasing due to reduced crop yields under recent detrimental climate changes. In particular, elevated temperature and limited irrigation in crop fields decreased appropriate cultivation areas for crops (Fahad et al., 2017). Changing environments such as increased CO_2 level and temperature affect crop susceptibility to pathogen attacks and crop production (Elad and Pertot, 2014; Gregory et al., 2009). Chinese cabbage, a major vegetable crop in several countries, is one of the crops encountering adverse agricultural environmental changes; high temperature and drought climate have limited the vegetative growth and seed yields in recent decades (Angadi et al., 2000; Lee et al., 2016; Oh et al., 2014; Sammis et al., 1988; Yan, 2015).

A variety of bacterial species interacts with plants in the rhizosphere and often mediates beneficial effects by promoting plant growth and activating plant defence responses

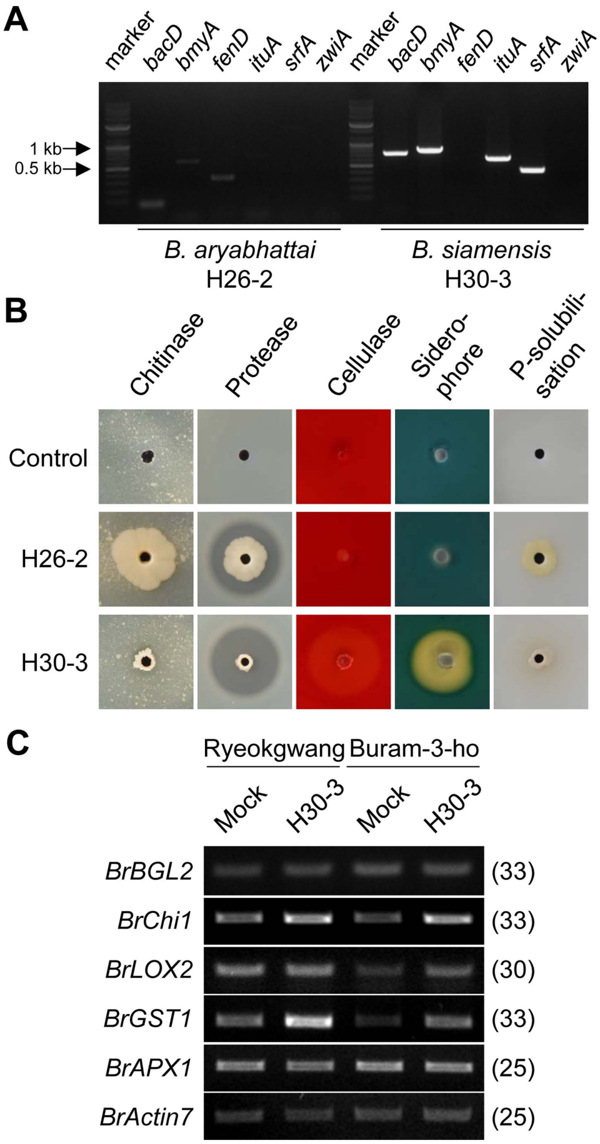


Fig. 5. Antifungal metabolites production and induced defence response of Chinese cabbage plants by *Bacillus siamensis* H30-3. (A) PCR-based detection of bacterial genes encoding antimicrobial lipopeptides from *B. aryabhattai* H26-2 and *B. siamensis* H30-3 genomes. M, DNA size marker. *bacD*, bacilysin; *bmyA*, bacillomycin; *fenD*, fengycin; *ituA*, iturin A; *srfA*, surfactin; *zwiA*, zwittermicin A. (B) Characteristics of hydrolytic enzyme secretion, siderophore production and phosphate (P)-solubilisation originated from *B. siamensis* H30-3. *In vitro* productions of chitinase, protease and cellulase by *B. siamensis* H30-3 were examined on different agar media described in Materials and methods. (C) Expression of defence-related genes in Chinese cabbage leaves treated with *B. siamensis* H30-3. Expression of basic glucanase 2 gene *BrBGL2*, chitinase 1 gene *BrChi1*, glutathione-*S*-transferase 1 gene *BrGST1* and ascorbate peroxidase 1 gene *BrAPX1* was analyzed by semi-quantitative RT-PCR technique. *BrActin7* was used as an internal control. The number of PCR cycles of each result is indicated within the right parenthesis.

against abiotic stresses and/or pathogen attacks, which lead to improved production in many economically important crops (Maksimov et al., 2011; Ngumbi and Kloepper, 2016; Yang et al., 2009; Yoo and Sang, 2017). Different *Bacillus* species have shown increased tolerance of various crops to environmental stresses including drought, high salinity and high temperature (Abd_Allah et al., 2017; Lim and Kim, 2013; Nautiyal et al., 2013; Park et al., 2017; Timmusk et al., 2014). Rhizobacteria-induced high temperature stress tolerance was only described in soybean plants, which was mediated by *B. aryabhattai* strain SRB02 (Park et al., 2017). Changes in phytohormones and abscisic acid-related gene expressions were demonstrated during the high temperature stress tolerance of soybean plants. In this study, two rhizobacteria *B. aryabhattai* H26-2 and *B. siamensis* H30-3 applied to two Chinese cabbage cultivars enhanced tolerance in response to high temperature stress. Heat stress led to various physiological and molecular changes in Brassicaceae plants. Differential glucosinolate volatiles were released in *Brassica nigra* plants in response to three different heat stress regimes (Kask et al., 2016). Highly activated antioxidant enzymes peroxidase and catalase were closely associated with heat tolerance of *B. juncea* plants (Wilson et al., 2014). Several genes encoding putative nucleotide-binding proteins showed higher expressions in heat-resistant *B. oleracea* line compared to those in heat-susceptible one (Kim et al., 2015c). Recently, differentially expressed genes were demonstrated in heat-susceptible and -tolerant Chinese cabbage genotypes in response to heat shock (Dong et al., 2015). However, no mechanism of rhizobacteria-mediated heat stress tolerance has been investigated in *Brassica* plants yet. Study of the biochemical and molecular changes in the Chinese cabbage plants during the two *Bacillus* strains-mediated stress tolerance to chronic high temperature is helpful to understand the mode-of-action in regulating the stress response.

B. aryabhattai H26-2 and *B. siamensis* H30-3 were themselves relatively more tolerant to high temperature and dehydration conditions compared to two phytopathogenic bacteria *Pcc* and *Xcc* (Fig. 2). 'Ryeokgwang' had relatively higher tolerance to high temperature compared to 'Buram-3-do' under greenhouse conditions, but no difference was found in drought tolerance (Fig. 1). *B. aryabhattai* H26-2 and *B. siamensis* H30-3 increased stress tolerance of two cultivars to high temperature, but not drought. No beneficial effect of the two rhizobacteria was found against combined stress of high temperature and drought stress (data not shown).

B. siamensis H30-3 pretreatment protected Chinese cabbage cv. 'Ryeokgwang' against black spot and anthracnose diseases (Fig. 4C). Crop protection by *B. siamensis* has not been described so far, although biological controls by various *Bacillus* species such as *B. subtilis*, *B. amyloliquefaciens* and *B. vallismortis* have been prevalently demonstrated in many crops (Shafi et al., 2017). Reduced black rot and anthracnose of the Chinese cabbage plants may result from production of many antifungal metabolites produced by the *B. siamensis* H30-3 (Fig. 5). Presence of cyclic lipopeptides biosynthetic gene homologues suggests that cyclic lipopeptides such as bacilysin, bacillomycin, iturin and surfactin may be involved in antifungal activity of the H30-3 strain against *A. brassicicola* and *C. higginsianum* (Ongena and Jacques, 2008; Shafi et al., 2017). Although homologous nucleotide sequences to cyclic lipopeptides biosynthetic genes were detected in the *B. siamensis* H30-3 genome, molecular structures and antifungal activities of cyclic lipopeptides secreted from the H30-3 strain remains elucidated. Secreting hydrolytic enzymes such as chitinase and protease from *B. siamensis* H30-3 might be involved in the induced crop protection of Chinese cabbage plants through the direct antifungal activities against the fungal cell walls and proteins (Alamri, 2015; Neeraja et al., 2010; Reyes-Ramírez et al., 2004). Capability to produce extracellular cellulase and siderophore was found in the *B. siamensis* H30-3, which suggests that the strain H30-3 may also apply to Chinese cabbage plants to cope with invading pathogens containing cellulose in their structures (e.g. *Hyaloperonospora parasitica*) and pathogens requiring iron for their growths (e.g. *Fusarium oxysporum* f. sp. *conglutinans*) (Ahmad et al., 2017; Duijff et al., 1993; Sherriff and Lucas, 1990; Slusarenko and Schlaich, 2003; Sulochana et al., 2014). Applications of the H30-3 to the Chinese cabbage for disease management of downy mildew and Fusarium wilt remains investigated.

Enhanced plant defence reactions by the *B. siamensis* H30-3 could not be excluded, because pathogenesis-related genes such as *BrChi1* and *BrGST1* were expressed in Chinese cabbage leaves pretreated with the strain H30-3 before the fungal challenge inoculation (Fig. 5C). It is supported by the facts that *BrChi1* and *BrGST1* gene expressions were pathogen-inducible in two Chinese cabbage cultivars by *C. higginsianum* and *A. brassicicola* infections in our previous study (Lee and Hong, 2014). Induced *BrChi1* expressions were found in the two Chinese cabbage cultivars with a similar level. *B. siamensis* H30-3 produced its own chitinase as well as mediated induced chitinase gene expression in Chinese cabbage plants. Highly inducible *BrGST1* gene expression might be more closely associated with the H30-3-meidated crop protection in 'Ryeokgwang' through glutathione-dependent detoxification and/or defence signalling activation (Dixon et al., 2002). Enhanced defence-related gene expressions by *Bacillus* species in tobacco plants were consistent with our present results (Kim et al., 2015a; Park et al., 2001; Wang et al., 2016). One or more of the cyclic lipopeptides or peptides secreted from the *B. siamensis* H30-3 can be molecular determinants for eliciting disease resistance of Chinese cabbage plants (Ongena et al., 2007; Wang et al., 2016). Recently, it was demonstrated that volatiles emitted by *Bacillus* spp. played roles in plant growth promotion and crop protection (Asari et al., 2016; Fincheira and Quiroz, 2018; Tahir et al., 2017). *B. siamensis* H30-3-mediated volatiles for disease resistance of Chinese cabbage plants remains elucidated as well.

Taken together, rhizobacteria *B. aryabhattai* H26-2 and *B. siamensis* H30-3 enhanced tolerance of Chinese cabbage seedlings to high temperature stress. *B. siamensis* H30-3 also showed protective efficacies on Chinese cabbage seedlings against *A. brassicicola* and *C. higginsianum* infections. The current study will provide more insights on the rhizobacteria-mediated crop protection against abiotic and biotic stresses in Chinese cabbage plants.

Acknowledgments

This research was supported by a grant from Program (grant no. PJ0125172018), Rural Development Administration, Republic of Korea.

References

Abd_Allah, E. F., Alqarawi, A. A., Hashem, A., Radhakrishnan, R., Al-Huqail, A. A., Al-Otibi, F. O. N., Malik, J. A., Alharbi, R. I. and Egamberdieva, D. 2017. Endophytic bacterium *Bacillus subtilis* (BERA 71) improves salt tolerance in chickpea plants by regulating the plant defense mechanisms. *J. Plant Interact.* 13:37-44.

Ahmad, Z., Wu, J., Chen, L. and Dong, W. 2017. Isolated *Bacillus subtilis* strain 330-2 and its antagonistic genes identified by the removing PCR. *Sci. Rep.* 7:1777.

Alamri, S. A. 2015. Enhancing the efficiency of the bioagent *Bacillus subtilis* JF419701 against soil-borne phytopathogens by increasing the productivity of fungal cell wall degrading enzymes. *Arch. Phytopathol. Plant Protect.* 48:159-170.

Ali, S. Z., Sandhya, V., Grover, M., Kishore, N., Venkateswar Rao, L. and Venkateswarlu, B. 2009. *Pseudomonas* sp. strain AKM-P6 enhances tolerance of sorghum seedlings to elevated temperatures. *Biol. Fertil. Soils* 46:45-55.

Ali, S. Z., Sandhya, V., Grover, M., Venkateswar Rao, L. and Venkateswarlu, B. 2011. Effect of inoculation with a thermotolerant plant growth promoting *Pseudomonas putida* strain AKMP7 on growth of wheat (*Triticum* spp.) under heat stress.

J. Plant Interact. 6:239-246.

Angadi, S. V., Cutforth, H. W., Miller, P. R., McConkey, B. G., Entz, M. H., Brandt, S. A. and Volkmar, K. M. 2000. Response of three *Brassica* species to high temperature stress during reproductive growth. *Can. J. Plant Sci.* 80:693-701.

Asari, S., Matzén, S., Petersen, M. A., Bejai, S. and Meijer, J. 2016. Multiple effects of *Bacillus amyloliquefaciens* volatile compounds: plant growth promotion and growth inhibition of phytopathogens. *FEMS Microbiol. Ecol.* 92:fiw070.

Asari, S., Ongena, M., Debois, D., De Pauw, E., Chen, K., Bejai, S. and Meijer, J. 2017. Insights into the molecular basis of biocontrol of *Brassica* pathogens by *Bacillus amyloliquefaciens* UCMB5113 lipopeptides. *Ann. Bot.* 120:551-562.

Dixon, D. P., Lapthorn, A. and Edwards, R. 2002. Plant glutathione transferases. *Genome Biol.* 3:3004.1-3004.10.

Dong, X., Yi, H., Lee, J., Nou, I. S., Han, C. T. and Hur, Y. 2015. Global gene-expression analysis to identify differentially expressed genes critical for the heat stress response in *Brassica rapa*. *PLoS One* 10:e0130451.

Duijff, B. J., Meijer, J. W., Bakker, P. A. H. M. and Schippers, B. 1993. Siderophore-mediated competition for iron and induced resistance in the suppression of fusarium wilt of carnation by fluorescent *Pseudomonas* spp. *Neth. J. Plant Pathol*. 99:277-289.

Elad, Y. and Pertot, I. 2014. Climate change impacts on plant pathogens and plant diseases. *J. Crop Improv.* 28:99-139.

Fahad, S., Bajwa, A. A., Nazir, U., Anjum. S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M. Z., Alharby, H., Wu, C., Wang, D. and Huang, J. 2017. Crop production under drought and heat stress: plant responses and management options. *Front. Plant Sci.* 8:1147.

Fincheira, P. and Quiroz, A. 2018. Microbial volatiles as plant growth inducers. *Microbiol. Res.* 208:63-75.

Gregory, P. J., Johnson, S. N., Newton, A. C. and Ingram, J. S. 2009. Integrating pests and pathogens into the climate change/food security debate. *J. Exp. Bot.* 60:2827-2838.

Han, J.-H., Shim, H., Shin, J.-H. and Kim, K. S. 2015. Antagonistic activities of *Bacillus* spp. strains isolated from tidal flat sediment towards anthracnose pathogens *Colletotrichum acutatum* and *C. gloeosporioides* in South Korea. *Plant Pathol. J.* 31:165-175.

Han, J.-H., Park, G.-C. and Kim, K. S. 2017. Antagonistic evaluation of *Chromobacterium* sp. JH7 for biological control of ginseng root rot caused by *Cylindrocarpon destructans*. *Mycobiololgy* 45:370-378.

Jeong, H., Jeong, D.-E., Kim, S. H., Song, G. C., Park, S.-Y., Ryu, C.-M., Park, S.-H. and Choi, S.-K. 2012. Draft genome sequence of the plant growth-promoting bacterium *Bacillus siamensis* KCTC 13613^T. *J. Bacteriol.* 194:4148-4149.

Kakar, K. U., Ren, X.-L., Nawaz, Z., Cui, Z.-Q., Li, B., Xie, G.-L., Hassan, M. A., Ali, E. and Sun, G.-C. 2016. A consortium of rhizobacterial strains and biochemical growth elicitors improve cold and drought stress tolerance in rice (*Oryza sativa* L.). *Plant Biol.* 18:471-483.

Kang, S.-M., Radhakrishnan, R. and Lee, I. J. 2015. *Bacillus amyloliquefaciens* subsp. *plantarum* GR53, a potent biocontrol agent resists *Rhizoctonia* disease on Chinese cabbage through hormonal and antioxidants regulation. *World J. Microbiol. Biotechnol.* 31:1517-1527.

Kask, K., Kännaste, A., Talts, E., Copolovici, L. and Niinemets, Ü. 2016. How specialized volatiles respond to chronic and short-term physiological and shock heat stress in *Brassica nigra*. *Plant Cell Environ.* 39:2027-2042.

Kavamura, V. N., Santos, S. N., da Silva, J. L., Parma, M. M., Ávila, L. A., Visconti, A., Zucchi, T. D., Taketani, R. G., Andreote, F. D. and de Melo, I. S. 2013. Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. *Microbiol. Res.* 168:183-191.

Kim, B.-R., Park, M.-S., Han, K.-S., Hahm, S.-S., Park, I.-H. and Song, J.-K. 2018. Biological control using *Bacillus toyonensis* strain CAB12243-2 against soft rot on Chinese cabbage. *Korean J. Organic Agric.* 26:129-140.

Kim, J.-S., Lee, J., Lee, C.-H., Woo, S. W., Kang, H., Seo, S.-G. and Kim, S.-H. 2015a. Activation of pathogenesis-related genes by the rhizobacterium, *Bacillus* sp. JS, which induces systemic resistance in tobacco plants. *Plant Pathol. J.* 31:195-201.

Kim, S. Y., Sang, M. K., Weon, H.-Y., Jeon, Y.-A., Ryoo, J. H. and Song, J. 2016. Characterization of multifunctional *Bacillus* sp. GH1-13. *Korean J. Pestic. Sci.* 20:189-196 (in Korean).

Kim, Y. J., Lee, Y. H., Lee, H.-J., Jung, H. and Hong, J. K. 2015b. H_2O_2 production and gene expression of antioxidant enzymes in kimchi cabbage (*Brassica rapa* var. *glabra* Regel) seedlings regulated by plant development and nitrosative stress-triggered cell death. *Plant Biotechnol. Rep.* 9:67-78.

Kim, Y.-W., Jung, H.-J., Park, J.-I., Hur, Y. and Nou, I.-S. 2015c. Response of NBS encoding resistance genes linked to both heat and fungal stress in *Brassica oleracea*. *Plant Physiol. Biochem.* 86:130-136.

Lee, S. G., Lee, H. J., Kim, S. K., Choi, C. S., Park, S. T., Jang, Y. A. and Do, K. R. 2015. Effects of vernalization, temperature, and soil drying periods on the growth and yield of Chinese cabbage. *Korean J. Hortic. Sci. Technol.* 33:820-828.

Lee, S. G., Lee, H. J., Kim, S. K., Choi, C. S. and Park, S. T. 2016. Influence of waterlogging period on the growth, physiological responses, and yield of kimchi cabbage. *J. Environ. Sci. Int.* 25:535-542 (in Korean).

Lee, Y. H. and Hong, J. K. 2012. Host and non-host resistance of kimchi cabbage against different *Xanthomonas campestris* pathovars. *Plant Pathol. J.* 28:322-329.

Lee, Y. H. and Hong, J. K. 2014. Differential defence responses of susceptible and resistant kimchi cabbage cultivars to anthracnose, black spot and black rot diseases. *Plant Pathol.* 64:406-415.

Lim, J.-H. and Kim, S.-D. 2013. Induction of drought stress resistance by multi-functional PGPR *Bacillus licheniformis* K11 in pepper. *Plant Pathol. J.* 29:201-208.

Maksimov, I. V., Abizgil'dina, R. R. and Pusenkova, L. I. 2011. Plant growth promoting rhizobacteria as alternative to chemical crop protectors from pathogens (Review). *Appl. Biochem. Microbiol.* 47:333-345.

Nautiyal, C. S., Srivastava, S., Chauhan, P. S., Seem, K., Mishra, A. and Sopory, S. K. 2013. Plant growth-promoting bacteria Bacillus amyloliquefaciens NBRISN13 modulates gene expression profiles of leaf and rhizosphere community in rice during salt stress. *Plant Physiol. Biochem.* 66:1-9.

Neeraja, C., Anil, K., Purushotham, P., Suma, K., Sarma, P. V. S. R. N., Moerschbacher, B. M. and Podile, A. R. 2010. Biotechnological approaches to develop bacterial chitinases as a bioshield against fungal diseases of plants. *Crit. Rev. Biotechnol.* 30:231-241.

Ngumbi, E. and Kloepper, J. 2016. Bacterial-mediated drought tolerance: Current and future prospects. *Appl. Soil Ecol.* 105:109-125.

Oh, S., Moon, K. H., Son, I.-C., Song, E. Y., Moon, Y. E. and Koh, S. C. 2014. Growth, photosynthesis and chlorophyll fluorescence of Chinese cabbage in response to high temperature. *Korean J. Hort. Sci. Technol.* 32:318-329. (in Korean)

Ongena, M. and Jacques, P. 2008. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. *Trend Microbiol.* 16:115-125.

Ongena, M., Jourdan, E., Adam, A., Paquot, M., Brans, A., Joris, B., Arpigny, J.-L. and Thonart, P. 2007. Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environ. Microbiol*. 9:1084-1090.

Park, K., Ahn, I.-P. and Kim, C.-H. 2001. Systemic resistance and expression of the pathogenesis-related genes mediated by the plant growth-promoting rhizobacterium *Bacillus amyloliquefaciens* EXTN-1 against anthracnose disease in cucumber. *Mycobiology* 29:48-53.

Park, T. -H., Choi, B. -S., Choi, A. -Y., Choi, I. -Y., Heu, S. and Park, B. -S. 2012. Genome sequence of *Pectobacterium carotovorum* subsp. *carotovorum* strain PCC21, a pathogen causing soft rot in Chinese cabbage. *J. Bacteriol.* 194:6345-6346.

Park, Y.-G., Mun, B.-G., Kang, S.-M., Hussain, A., Shahzad, R., Seo, C.-W., Kim, A.-Y., Lee, S.-U., Oh, K. Y., Lee, D. Y., Lee, I.-J. and Yun, B. W. 2017. *Bacillus aryabhattai* SRB02 tolerates oxidative and nitrosative stress and promotes the growth of soybean by modulating the production of phytohormones. *PLoS One* 12:e0173203.

Rakotoniriana, E. F., Rafamantanana, M., Randriamampionona, D., Rabemanantsoa, C., Urveg-Ratsimamanga, S., El Jaziri, M., Munaut, F., Corbisier, A.-M., Quetin-Leclercq, J. and Declerck, S. 2013. Study in vitro of the impact of endophytic bacteria isolated from *Centella asiatica* on the disease incidence caused by the hemibiotrophic fungus *Colletotrichum higginsianum. Antonie Van Leeuwenhoek* 103:121-133.

Reyes-Ramírez, A., Escudero-Abarca, B. I., Aguilar-Uscanga, G., Hayward-Jones, P. M. and Barboza-Corona, J. E. 2004. Antifungal activity of *Bacillus thuringiensis* chitinase and its potential for the biocontrol of phytopathogenic fungi in soybean seeds. *J. Food Sci.* 69:M131-M134.

Sammis, T. W., Kratky, B. A. and Wu, I. P. 1988. Effects of limited irrigation on lettuce and Chinese cabbage yields. *Irrigation Sci.* 9:187-198.

Sang, M. K., Dutta, S. and Park, K. 2015. Influence of commercial antibiotics on biocontrol of soft rot and plant growth promotion in Chinese cabbages by *Bacillus vallismortis* EXTN-1 and BS07M. *Res. Plant Dis.* 21:255-260.

Shafi, J., Tian, H. and Ji, M. 2017. *Bacillus* species as versatile weapons for plant pathogens: a review. *Biotechnol. Biotechnol. Equip.* 31:446-459.

Sherriff, C. and Lucas, J. A. 1990. The host range of isolates of downy mildew, *Peronospora parasitica*, from *Brassica* crop species. *Plant Pathol.* 39:77-91.

Slusarenko, A. J. and Schlaich, N. L. 2003. Downy mildew of Arabidopsis thaliana caused by *Hyaloperonospora parasitica* (formerly *Peronospora parasitica*). *Mol. Plant Pathol.* 4:159-170.

Sulochana, M. B., Jayachandra, S. Y., Kumar, S. K. A. and Dayanand, A. 2014 Antifungal attributes of siderophore produced by the *Pseudomonas aeruginosa* JAS-25. *J. Basic Microbiol.* 54:418-424.

Sun, C., Johnson, J. M., Cai, D., Sherameti, I., Oelmüller, R. and Lou, B. 2010. *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. *J. Plant Physiol.* 167:1009-1017.

Tahir, H. A. S., Gu, Q., Wu, H., Niu, Y., Huo, R. and Gao, X. 2017. *Bacillus* volatiles adversely affect physiology and ultrastructure of *Ralstonia solanacearum* and induce systemic resistance in tobacco against bacterial wilt. *Sci. Rep.* 7:40481.

Templer, S. E., Ammon, A., Pscheidt, D., Ciobotea, O., Schuy, C., McCollum, C., Sonnewald, U., Hanemann, A., Förster, J., Ordon, F., von Korff, M. and Voll, L. M. 2017. Metabolite profiling of barley flag leaves under drought and combined heat and drought stress reveals metabolite QTLs for metabolites associated with antioxidant defense. *J. Exp. Bot.* 68:1697-1713.

Timmusk, S., Abd El-Daim, I. A., Copolovici, L., Tanilas, T., Kännaste, A., Behers, L., Nevo, E., Seisenbaeva, G., Stenström, E. and Niinemets, Ü. 2014. Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: Enhanced biomass production and reduced emissions of stress volatiles. *PLoS One* 9:e96086.

Wang, C.-J., Yang, W., Wang, C., Gu, C., Niu, D.-D., Liu, H.-X., Wang, Y.-P. and Guo, J.-H. 2012. Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. *PLoS One* 7:e52565.

Wang, N., Liu, M., Guo, L., Yang, X. and Qiu, D. 2016. A novel protein elicitor (PeBA1) from *Bacillus amyloliquefaciens* NC6 induces systemic resistance in tobacco. *Int. J. Biol. Sci.*

12:757-767.

Wilson, R. A., Sangha, M. K., Banga, S. S., Atwal, A. K. and Gupta, S. 2014. Heat stress tolerance in relation to oxidative stress and antioxidants in *Brassica juncea*. *J. Environ. Biol.* 35:383-387.

Yan, M. 2015. Seed priming stimulate germination and early seedling growth of Chinese cabbage under drought stress. *S. Afr. J. Bot.* 99:88-92.

Yang, Y.-W., Tsai, C.-C., Liou, T.-D. and Chen, K.-S. 2001. Two heat-tolerant F1 hybrids of Chinese cabbage. *HortScience* 36:1144-1145.

Yang, J., Kloepper, J. W. and Ryu, C.-M. 2009. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci.* 14:1-4.

Yoo, S.-J. and Sang, M. K. 2017. Induced systemic tolerance to multiple stresses including biotic and abiotic factors by rhizobacteria. *Res. Plant Dis.* 23:99-113 (in Korean).

Zandalinas, S. I., Rivero, R. M., Martínez, V., Gómez-Cadenas, A. and Arbona, V. 2016. Tolerance of citrus plants to the combination of high temperatures and drought is associated to the increase in transpiration modulated by a reduction in abscisic acid levels. *BMC Plant Biol.* 16:105.

Zhao, F., Zhang, D., Zhao, Y., Wang, W., Yang, H., Tai, F., Li, C. and Hu, X. 2016. The difference of physiological and proteomic changes in maize leaves adaptation to drought, heat and combined both stresses. *Front. Plant Sci.* 7:1471.

Zhou, R., Yu, X., Ottosen, C.-O., Rosenqvist, E., Zhao, L., Wang, Y., Yu, W., Zhao, T. and Wu, Z. 2017. Drought stress had a predominant effect over heat stress on three tomato cultivars subjected to combined stress. *BMC Plant Biol.* 17:24.