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BURKHOLDERIA SP. KCTC 11096BP MODULATES PEPPER GROWTH AND RESISTANCE AGAINST *PHYTOPHTHORA CAPSICI*

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Abstract

Biological control of crop diseases is desirable for sustainable agriculture as it minimizes chemical inputs in the agricultural system and promotes eco-friendly environment. We analyzed the favorable role of *Burkholderia* sp. KCTC 11096BP against the pathogen *Phytophthora capsici* in pepper. We screen thirty rhizobateria for their anti-pathogen activity, and found that *Burkholderia* sp. KCTC 11096BP exhibits maximum growth inhibition of the pathogen *P. capsici*. The bacterium inoculation to pepper plants significantly enhanced growth attributes of pepper in infected and control treatments. The total proteins (10.9%), and the amino acids viz. glycine (4.08 ug/g), leucine (3.3 ug/g), and alanine (3.26 ug/g) were preset in considerably higher quantities in *Burkholderia* sp. applied treatments as compare to control. The systemic acquired resistance (SAR) of the host plant was up-regulated by *Burkholderia* sp. KCTC, as endogenous salicylic acid (235.5 ng/g) and jasmonic acid (22.8 ng/g) levels were found higher in such treatments. It was concluded that *Burkholderia* sp. KCTC 11096BP mitigates the adverse effects of *P. capsici* on pepper crop and can improve crop productivity at the field level.

Key words: *Burkholderia* sp. KCTC 11096BP, *Phytophthora capsici*, Pepper, Plant protection.

Introduction

Rhizosphere contains diverse microflora, which improve the performance of plants and aid to plant growth and development by one way or another. A group of rhizoshere inhabiting bacteria have been recently known to confor wide array of tollerance to plants during unfavourable growth conditions (Baniaghil *et al.*, 2013; Gou *et al*., 2015). These plant growth promoting rhizobacteria (PGPR) helps the plant in acquisation of minerals, whilst the plant provide a protective sanctury for their reproduction and food through exudation of secondary metabolities (Bano *et al.*, 2013; Verma *et al.*, 2013). In return, PGPR offers resistance to both biotic and abiotic stresses by releasing bioactive metabolites and re-programming plant's responses against stress (Bhattacharyya & Jha, 2012; Khan *et al.*, 2013).

Biotic stress in the form of pathogenic attack creates a set of morphological and physiological impacts on plants ranging from biochemical to transcriptomic levels. Plant usually respond by activating its defence signalling in the form of increasing the generation of bioactive metabolites like jasmonic acid (JA) and salicylic acid (SA) and reprogramming its growth by regulating functional biochemicals like amino acids (Smith *et al.*, 2009).

Phytophthora species are a destructive group of filamentous plant pathogens, which have a global distribution and devastating effect on a wide range of plants important to agriculture and natural ecosystems (Fisher *et al.*, 2012). The economic impact of this group of pathogens remains the principal driving force behind the need to understand *Phytophthora* parasitism and epidemics. *Phytophthora* spp. are hemi-biotrophic pathogens, having a lifestyle that features a biotrophic phase, followed by a switch to necrotrophy (Judelson & Blanco, 2005). *P. capsici* is an oomycete plant pathogen that causes seed rot and seedling blight in many solanaceous crops (pepper, eggplant, tomato) and cucurbits (cantaloupe, cucumber, summer squash, pumpkin, watermelon), causing multi-billion dollar losses in crop production annually (Lamour *et al.*, 2012). The roots, stems, foliage, and fruit of mature pepper plants are susceptible. The pathogen induced infection can occur at any height on stems, it is most common at the soil line, and starts as a dark, water-soaked spot. The stem lesions become dark brown to black and results in girdling and plant death (Gevens *et al.*, 2008).

In current study, we studied the effects of *P. capsici* on pepper (*Capsicum annum* L.), and observed the role of the PGPR (*Burkholderia* sp. KCTC 11096BP) in rescuing pepper growth by mitigating the adverse effects of the pathogen on host. *Burkholderia* sp. KCTC 11096BP has been previously reported for production of bioactive secondary metabolites and phytohormones (Joo *et al.*, 2009; Kang *et al.*, 2012; Afzal *et al.,* 2016). In addition, the PGPR has been reported for its phosphate solubilization capacity and improving the plant growth by mediating phyto-hormonal responses. However, little is known about the role of PGPR in *P. capsici* infected pepper plants.

Material and Methods

Collection and isolation of PGPR: We collected rhizospheric soil samples from pepper fields in Andong, Chungju, Jinju, Sangju and Yecheon localities of

Gyeongbuk Province. The soil samples were thoroughly mixed and 10g from pooled soil was transferred to 250 ml flasks containing 100 ml of sterile Amies solution (Amies, 1967). Resulting suspensions were serially diluted (10^{-4}) and 0.1 ml aliquots were plated on tryptic soy agar (TSA; Merck Co., Germany), for isolation of microbes. The bacterial cultures were incubated and observed for 72h at 30°C, and bacterial colonies differentiated by their morphology, pigmentation and growth rate were isolated, counted and re-streaked on fresh TSA medium. For long term preservation, bacteria were stored in 50% glycerol at -20°C. The 30 bacterial isolates were screened for their growth inhibition of *P. capsici*. For screening bioassay, the bacterial culture suspension (CS) was grown in nutrient broth (NB composition gm L^{-1}, peptic digest of animal tissue 5.00, sodium chloride 5.00, beef extract 1.50, yeast extract 1.50, final pH (at 25°C), 7.4±0.2), and incubated for 3 days at 30°C on a shaking incubator set at 200 rpm, to obtain cell density of 108CFU/ml. The bacterial isolate SE4 recorded best inhibition of *P. capsici*, and was selected for further study.

Molecular identification of is isolate SE4: The bacterial isolate SE4 was identified as *Burkholderia* sp. KCTC 11096BP on the basis of sequences obtained for 16S rRNA region. The 16S rRNA was PCR amplified using the 27F primer (5′-AGAGTTTGATC(AC)TGGCTCAG-3′) and 1492R primer (5′-CGG (CT) TACCTTGTTACGACTT-3′), which were complementary to the 5′ end and 3′ end of the prokaryotic 16S rRNA, respectively (Adachi *et al*., 1996). The bacterial isolate SE4 used in current study was earlier reported for gibberellins production (Joo *et al*., 2009). The bacterial isolate was deposited to the Korea Collection for Type Cultures (KCTC) and was allotted no. KCTC 11096BP (Joo *et al*., 2009).

Bacterial isolate SE4 and capsicum growth: Pepper seeds were purchased from Seminis Korea Co. (Korea), surface sterilized with sodium hypochlorite (5%) for 10 min, and thoroughly rinsed with double distilled water. The seeds were sown in plastic pots containing horticultural soil under controlled greenhouse conditions (30±2ºC). The composition of horticultural soil was; peat moss (13-18%), perlite (7-11%), coco-peat (63-68%) and zeolite (6-8%), while the macro-nutrients were present as follows: NH_4+~90 mg/L; NO_3- ~205 mg/L; P_2O_5 ~350 mg/L and K_2O~100 mg/L (Khan *et al*., 2012).

Two-weeks old seedlings were treated with 5 ml ($x10^7$ CFU/ml) of bacterial CS and after one week of such treatment, the pepper plants were infected with *P. capsici* (obtained from KCTC, Korea). After three weeks of *P. capsici* application, the growth attributes i.e. shoot length, plant fresh and dry biomass, and leaves chlorophyll contents were recorded. The experiment designed comprised four treatments (four replicates per treatment and each replicate comprised 24 plants), and the experiment was repeated thrice. The chlorophyll contents of all fully expanded leaves were analyzed with Chlorophyll meter (Minolta Co., Ltd, Japan).

Endogenous salicylic acid (SA) and jasmonic acid (JA) analysis of pepper: SA was extracted and quantified as described previously by Seskar *et al.* (1998) . Briefly, freeze dried leaf samples were grinded to powder form and 0.1g was sequentially extracted with 90 and 100% methanol by centrifuging at 10,000×*g*. The combined methanol extracts was vacuum dried. Dry pellets were re-suspended in 2.5ml of 5% trichloroacetic acid and the supernatant was partitioned with ethyl acetate: cyclopentane: isopropanol (100:99:1, v/v). The top organic layer containing free SA was transferred to a 4ml vial and dried with nitrogen gas. The dry SA was again suspended in 1 ml of 70% methanol. High Performance Liquid Chromatography (HPLC) analysis were carried out on Shimadzu having fluorescence detector (Shimdzu RF-10AXL, excitation and emission 305-365 nm respectively) fitted with C18 reverse-phase HPLC column (HP hypersil ODS, particle size 5µm, pore size 120Å Waters). The flow rate was 1.0 ml/min.

The endogenous jasmonic acid (JA) level was extracted according to the protocol of McCloud and Baldwin (1997). The extracts were analyzed with GC-MS SIM (6890N network GC system, and 5973 network mass selective detector; Agilent Technologies, Palo Alto, CA, USA). To enhance the sensitivity of the method, spectra were recorded in the selected ion mode i.e. in case of JA determination, monitored the fragment ion at *m/z*= 83 amu corresponding to the base peaks of JA and [9, 10-$^2H_2$]-9, 10-dihydro-JA. The amount of endogenous JA was calculated from the peak areas of endogenous JA in compared with the corresponding standards. Three replicates per treatment were used for determination of SA and JA from three independent experiments.

Analysis of pepper proteins and amino acids: The nitrogen contents of pepper leaves were estimated following Kjeldahl method (1883), using a nitrogen auto-analyzer (Tecator Kjeltec Auto Analyzer Model 1030) and crude protein contents were calculated (% N×6.25).

Pepper plants from all treatments were immediately frozen in liquid nitrogen and then stored at -80°C. The leaves were freeze dried in Virtis Freeze Dryer (-55 °C; Gardiner, NY, USA), and the powdered leaves were hydrolyzed with 6 M HCl at 110 °C for 24 h, in hydrolysis tubes. The resulting product was dried, dissolved in 0.02 N HCl and centrifuged at 10,000 rpm for 15 min. The amino acid analyzer (HITACHI L-8900, Japan) attached with HITACHI HPLC packed column containing ion-exchanging resin No. 2622 PF (4.6 × 60 mm) and UV detector (VIS1: 570 nm, VIS2: 440 nm), was used for the analysis of amino acids. Wako L-8500 buffer solution PF-1, 2, 3, 4 and RG were used in current study and 20-µl of each sample was injected.

Statistical analysis: The differences among the mean values were determined using Duncan's multiple range tests (DMRT) at $p<0.05$. The results were graphically presented using Graph Pad Prism software (version 5.0, San Diego, California USA), while Statistic Analysis System (SAS 9.1) was used for DMRT analysis.

Results

Bioassay for identifying *P. capsici* inhibiting novel PGPR: The bacterial isolates collected from rhizospheric soil samples, were grown on *P. capsici* containing agar plates. Results showed that bacterial isolate SE4 significantly inhibited the growth of *P. capsici* as compared to other PGPR isolates (Table 1 and Fig. 1a). Some other bacterial isolates SE34, SE490, SE637, SE691 and SE709, also inhibited the growth of *P. capsici* but their effects were much lesser than SE4 (Table 1).

Table 1. Growth inhibitory activity of isolated strains against the *P. capsici*.

Strains	Inhibitive activity	Strains	Inhibitive activity
SE4	+ + +	SE489	+ +
SE24	+ +	SE490	+ +
SE27	+ +	SE534	+
SE34	+ +	SE535	+ +
SE55	+	SE569	+
SE85	+ +	SE637	+ +
SE130	+	SE670	+ +
SE138	+	SE687	+ +
SE151	+	SE689	+
SE201	+ +	SE691	+ +
SE231	+ +	SE697	+
SE234	+	SE703	+ +
SE320	+	SE709	+ +
SE326	+ +	SE712	+
SE358	+	SE786	+ +

+++ shows significant/strong growth inhibitory; ++ moderate growth inhibitory; + neutral or week growth inhibitory. The experiment was independently repeated three times (n=15)

Bacterial isolate SE4 and pepper plant growth: The isolate SE4 significantly promoted growth attributes of peeper and mitigated the adverse effects of *P. capsici* (Table 2). The maximum shoot length (28.28 cm), plant fresh biomass (7.68 gm), plant dry biomass (0.98 gm) and no. of leaves (19.4) were recorded for SE4 applied treatments. The plants treated with both SE4 and *P. capsici* produced second best results, while least growth attributes were observed in sole *P. capsici* treated plants as compared to control (Table 2 and Fig. 1b). Current results clearly demonstrate the favorable role of SE4 in checking the adverse effects of the pathogen.

Analysis of pepper total proteins and essential amino acids: Our results showed that the total protein contents were significantly higher in SE4+ *P. capsici* applied treatments (10.9%), SE4 treatments (9.8%), and while least in sole *P. capsici* treated plants (Fig. 2). Similarly, the amino acid levels were also higher in SE4 (33.51 ug/g), SE4+ *P. capsici* (15.15 ug/g), while least (4.34 ug/g) in sole *P. capsici* treated plants. The glycine (4.08 ug/g), leucine (3.3 ug/g), and alanine (3.26 ug/g) contents were considerably higher in *Burkholderia* sp. KCTC treated pepper as compared to control (Table 3).

Jasmonic acid (JA) and Salicylic acid (SA) analysis: JA and SA are important player of systemic acquired resistance (SAR). Current study revealed that endogenous JA and SA levels were considerably higher in plants treated with SE4+ *P. capsici* i.e. 22.8 ng/g and 235.5 ng/g respectively. The endogenous JA and SA levels were higher in pepper plants treated with sole SE4 (19.3 and 64 ng/g respectively), and *P. capsici* (16.4 and 52.4 ng/g respectively) as compared to control (Fig. 3).

Fig. 1. (a) Pure culture of bacterial isolate (b) Effect of bacterial isolate and pathogen on pepper seedlings.

Table 2. Effect of PGPR application to pepper plants in avoiding pathogenic attack and effect on growth attributes.

Treatments		C	P	B	B + P
Length (cm)	Shoot	23.64 ± 0.88^{b}	20.7 ± 0.67^{c}	28.28 ± 0.67^{a}	27.26 ± 0.72^{ab}
	Root	2.3 ± 0.16^{b}	1.74 ± 0.37^{c}	2.84 ± 0.11^{a}	2.46 ± 0.22^{b}
Fresh weight(g)	Shoot	3.36 ± 0.30^{b}	2.66 ± 0.24^{c}	4.58 ± 0.37^{a}	3.9 ± 0.19^{b}
	Root	1.9 ± 0.27^{c}	1.1 ± 0.27^{d}	3.1 ± 0.27^{a}	2.78 ± 0.13^{b}
Dry weight(g)	Shoot	0.42 ± 0.02^{b}	0.33 ± 0.03^{c}	0.62 ± 0.03^{a}	0.58 ± 0.03^{ab}
	Root	0.24 ± 0.03^{c}	0.12 ± 0.02^{d}	0.36 ± 0.03^{a}	0.30 ± 0.02^{b}
No. of leaves		14.2 ± 0.84^{c}	12.4 ± 0.55^{d}	19.4 ± 0.55^{a}	17.6 ± 0.55^{b}
Chlorophyll (SPAD)		25.7 ± 0.77^{b}	23.02 ± 0.58^{c}	33.78 ± 1.91^{a}	31.56 ± 1.11^{ab}

The experiment was repeated three times independently. ±; S.E, C; Control, B; *Burkholderia* sp. KCTC 11096BP SE4, P; *Phytophthora capsici*

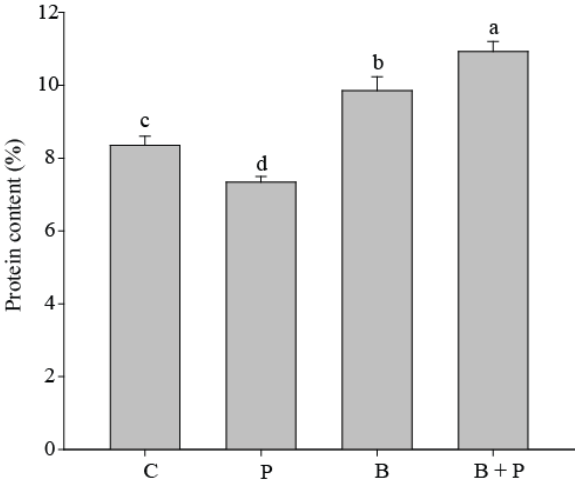

Fig. 2. Effect of PGPR application and pathogenic attack on the total protein content of the pepper plants. Total protein content was determined in pepper leaves from three independently experiments and SE was worked out.

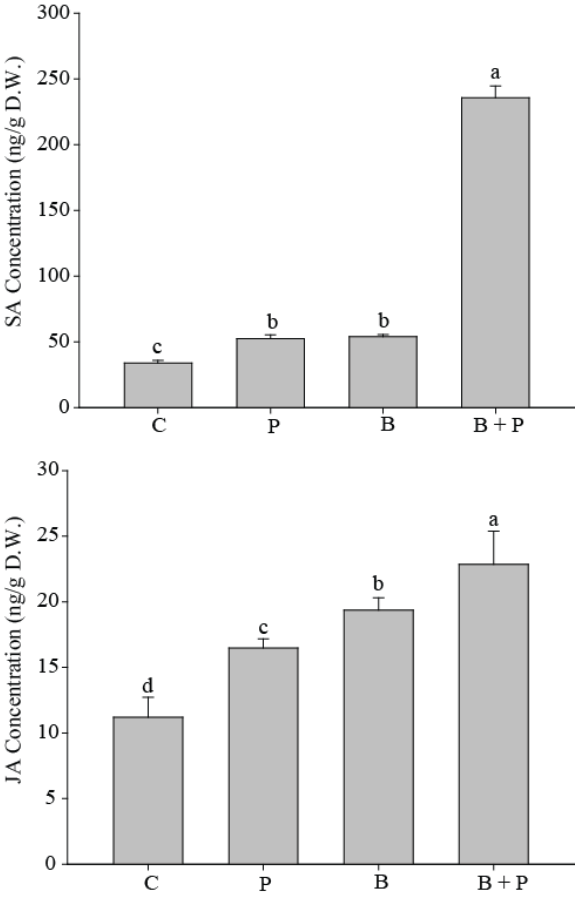

Fig. 3. Effect of PGPR application (*Burkholderia* sp. KCTC 11096BP) and pathogenic attack (*P. capsici*) on the endogenous SA and JA contents of the pepper plants. For each set of treatment, the different letter indicates significant differences ($p<0.05$) between PGPR and control as evaluated by Duncan multiple range test. ± refers to SE of the mean of three readings per treatment from experiment repeated three times independently.

Discussion

Plant growth promoting rhizobacteria (PGPR) have been reported to ameliorate crop growth under stress environments like salinity, drought, heat, and heavy metal etc. (Hamayun et al., 2010; Aboudrar *et al.*, 2013; Baniaghil *et al.*, 2013; Bano *et al.*, 2013; Botta *et al.*, 2013; Joo*, et al.*, 2009; Kang *et al.*, 2010; Kang *et al.*, 2009; Singh *et al.*, 2013; Kiani *et al*., 2016). However, little is known about their favorable role in mitigating the adverse impact of plant pathogens. In present study, we observed that *Burkholderia* sp. KCTC 11096BP significantly rescued plant growth and mitigated the adverse effects of *P. capsici* on pepper crop. *P. capsici* is a well-known plant pathogen and causes huge losses to pepper crop each year around the world. Similar observations were reported by Lee *et al*. (2013), where *Paenibacillus polymyxa* E681 induced systemic resistance against *Phytophthora* blight of pepper plants. The PGPR involved in current study was previously reported for the production of gibberellins (GAs) (Joo*, et al.*, 2009), which are known for regulating plant growth under normal and stress conditions (Khan *et al.*, 2012). The possible mechanism for enhancing disease resistance is plant growth promotion due to the phytohormone production ability of PGPR, as an increase in plant growth can modulate a resistance to a variety of abiotic and biotic stresses (Kuldau & Bacon, 2008).

The plant growth promotion in our study could be easily attributed to the GA production capacity of the bacterial isolate SE4. However, an up-regulation of plant amino acids and endogenous plant hormones mitigated the adverse impact of *P. capsici*, as the PGPR strengthened the pepper immune system by activating certain disease specific secondary metabolites. Our current findings confirm previous report, which observed that activation in amino acid contents by PGPR during biotic elicitation has been found to enhance the resistance of the plant (Bhattacharyya & Jha, 2012). Sophearetha *et al.* (2013) reported that the negative impacts of pathogenic attacks were minimized, as *Burkholderia cepacia* MPC-7 enhanced the disease resistance capacity of pepper plants.

Pathogenic attacks on plants activate broader array of signaling molecules in order to strengthen resistance of the host plant. JA and SA are known to trigger and mediate plant responses against such pathogenic attacks. We observed that the endogenous JA contents were higher in SE4+ *P. capsici* treatments, which suggest an activation of defense responses against *Phytophthora* infection (Smith *et al.*, 2009). Previously, Pieterse *et al.* (2002) reported that in Arabidopsis, JA induced long-term systemic defense responses, deemed rhizobacteria-induced systemic response (ISR), which was similar to SA-mediated systemic acquired resistance (SAR). SA activates SAR against pathogenic attacks in infected plant parts (Ryals *et al.*, 1996). However, in mutualistic relationship, SA production initiates induced systemic resistance (ISR) and improve plant performance under both biotic and abiotic stresses (Khan *et al.*, 2012; Khan *et al.*, 2011; Pozo & Azcón-Aguilar, 2007). Similarly, Alonso-Ramírez *et al.* (2009) reported that GA-responsive gene and exogenous addition of GAs

counteract the inhibitory effects of different adverse environmental conditions in seed germination and seedling growth of *Arabidopsis* through modulation of SA biosynthesis. The SA-initiated responses to pathogens in-turns also mediates excursion of hypersensitive responses causing the production of phytoalexins (Smith *et al.*, 2009). In current study, significantly higher endogenous SA contents in SE4+ *P. capsici* treatments suggested an initiation and strengthening of induced systemic resistance of the host plants. Current study confirms previous report of Umashankari & Sekar (2011), who observed that *P. oryzae pathosystem* can induce ISR in rice. An enhanced SA levels induce ISR into the plants, and help the plant to mitigate the adverse effects of pathogenic infection. Current study suggests a complex network of cross-talk between the SA and JA biosynthesis pathways, which fine tunes plant defense responses and mitigates the biotic stress induced by the pathogen.

Table 3. Effect of PGPR and pathogenic infection on the essential amino acids of pepper plants (Quantities in nmol DW).

Amino acids	C	P	B	B + P
Asp	1.16 ± 0.29	0.56 ± 0.09	2.00 ± 0.12	0.96 ± 0.10
Thr	0.46 ± 0.08	0.31 ± 0.07	2.03 ± 0.11	0.87 ± 0.09
Ser	0.6 ± 0.05	0.44 ± 0.08	2.15 ± 0.14	0.97 ± 0.10
Glu	0.69 ± 0.04	0.11 ± 0.02	2.50 ± 0.20	1.21 ± 0.15
Pro	1.05 ± 0.24	0.76 ± 0.14	2.10 ± 0.31	0.93 ± 0.07
Gly	0.48 ± 0.04	0.23 ± 0.01	4.08 ± 0.72	1.97 ± 0.11
Ala	0.99 ± 0.10	0.30 ± 0.06	3.26 ± 0.54	1.35 ± 0.09
Cys	0.13 ± 0.01	0.08 ± 0.01	0.14 ± 0.01	0.09 ± 0.01
Val	0.72 ± 0.02	0.27 ± 0.01	2.44 ± 0.21	1.05 ± 0.13
Met	0.1 ± 0.01	0.01 ± 0.01	0.07 ± 0.01	0.03 ± 0.01
Ile	0.47 ± 0.02	0.16 ± 0.02	1.74 ± 0.31	0.74 ± 0.04
Leu	0.62 ± 0.03	0.15 ± 0.01	3.30 ± 0.48	1.34 ± 0.14
Tyr	0.34 ± 0.04	0.07 ± 0.01	0.93 ± 0.03	0.50 ± 0.04
Phe	0.35 ± 0.01	0.12 ± 0.01	1.61 ± 0.35	0.74 ± 0.02
Lys	0.37 ± 0.02	0.41 ± 0.02	2.85 ± 0.30	1.36 ± 0.11
His	0.26 ± 0.01	0.22 ± 0.02	0.81 ± 0.01	0.40 ± 0.01
Arg	0.54 ± 0.02	0.14 ± 0.01	1.50 ± 0.01	0.65 ± 0.03
Total	9.33 ± 0.25^{c}	4.34 ± 0.11^{d}	33.51 ± 0.42^{a}	15.15 ± 0.34^{b}

C: Control, B: *Burkholderia* sp. KCTC 11096BP SE4, P: *Phytophthora capsici.* The experiment was repeated three times independently

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References

Aboudrar, W., C. Schwartz, J.L. Morel and A. Boularbah. 2013. Effect of nickel-resistant rhizosphere bacteria on the uptake of nickel by the hyperaccumulator *Noccaea caerulescens* under controlled conditions. *J. Soils Sediments*, 13: 501-507.

Adachi, M., Y. Sako and Y. Ishida. 1996. Analysis of *Alexandrium* (Dinophyceae) species using sequences of the 5.8S ribosomal DNA and internal transcribed spacer regions. *J. Phycol.,* 32: 424-432.

Afzal, I., I. Iqrar, Z.K. Shinwari and A. Yasmin. 2016. Plant growth-promoting potential of endophytic bacteria isolated from roots of wild *Dodonaea viscosa* L. *Plant Growth Regul.*,1-10: http://link.springer.com/article/10.1007/s10725-016-0216-5

Alonso-Ramírez, A., D. Rodríguez, D. Reyes, J.A. Jiménez, G. Nicolás, M. López-Climent, A. Gómez-Cadenas and C. Nicolás. 2009. Evidence for a role of gibberellins in salicylic acid-modulated early plant responses to abiotic stress in Arabidopsis seeds. *Plant Physiol.*, 150: 1335-1344.

Amies, C. 1967. A modified formula for the preparation of Stuart's transport medium. *Canad. J. Pub. Health*, 296-300.

Baniaghil, N., M. Arzanesh, M. Ghorbanli and M. Shahbazi. 2013. The effect of plant growth promoting rhizobacteria on growth parameters, antioxidant enzymes and microelements of canola under salt stress. *J. Appl. Environ. Biol. Sci.*, 3: 17-27.

Bano, Q., N. Ilyas, A. Bano, N. Zafar, A. Akram and F. Hassan. 2013. Effect of *Azospirillum* inoculation on maize (*Zea mays* L.) under drought stress. *Pak. J. Bot.*, 45: 13-20.

Bhattacharyya, P. and D. Jha. 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J. Microb. Biot.*, 28: 1327-1350.

Botta, A.L., A. Santacecilia, C. Ercole, P. Cacchio and M. Del Gallo. 2013. In vitro and in vivo inoculation of four endophytic bacteria on *Lycopersicon esculentum*. *New Biotechnol.*, 30: 666-674.

Fisher, M.C., D.A. Henk, C.J. Briggs, J.S. Brownstein, L.C. Madoff, S.L. McCraw and S.J. Gurr. 2012. Emerging fungal threats to animal, plant and ecosystem health. *Nature*, 484: 186-194.

Gevens, A.J., P.D. Roberts, R. McGovern and T. Kucharek. 2008. Vegetable diseases caused by *Phytophthora capsici* in Florida. *Plant Pathology Department document SP159. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida*,

Gou, W., L. Tian, Z. Ruan, P. Zheng, F. Chen, L. Zhang, Z. Cui, P. Zheng, Z. Li, M. Gao, W. Shi, L. Zhang, J. Liu and J. HU. 2015. Accumulation of choline and glycinebetaine and

drought stress tolerance induced in maize (*Zea mays*) by three plant growth promoting rhizobacteria (PGPR) strains. P*ak. J. Bot*., 47(2): 581-586.

Hamayun, M., S.A. Khan, A.L. Khan, Z.K. Shinwari, I. Iqbal, E.-Y. Sohn, M.A. Khan and I-J Lee. 2010. Effect of salt stress on growth attributes and endogenous growth hormones of soybean cultivar Hwangkeumkong. *Pak. J. Bot*., 42(5): 3103-3112

Joo, G.-J., S.-M. Kang, M. Hamayun, S.-K. Kim, C.-I. Na, D.-H. Shin and I.-J. Lee. 2009. *Burkholderia* sp. KCTC 11096BP as a newly isolated gibberellin producing bacterium. *J. Microbiol*., 47: 167-171.

Judelson, H.S. and F.A. Blanco. 2005. The spores of Phytophthora: weapons of the plant destroyer. *Nat. Rev. Microbiol*, 3: 47-58.

Kang, S.-M., M. Hamayun, G.-J. Joo, A. L. Khan, Y.-H. Kim, S.-K. Kim, H.-J. Jeong and I.-J. Lee. 2010. Effect of *Burkholderia* sp. KCTC 11096BP on some physiochemical attributes of cucumber. *Eur. J. Soil Biol*., 46: 264-268.

Kang, S.-M., G.-J. Joo, M. Hamayun, C.-I. Na, D.-H. Shin, H. Y. Kim, J.-K. Hong and I.-J. Lee. 2009. Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. *Biotecnol. Lett*., 31: 277-281.

Kang, S.-M., A.L. Khan, M. Hamayun, Z.K. Shinwari, Y.-H. Kim, G.-J. Joo and I.J. Lee. 2012. *Acinetobacter calcoaceticus* ameliorated plant growth and influenced gibberellins and functional biochemicals. *Pak. J. Bot.*, 44: 365-372.

Khan, A.L., M. Hamayun, S.-M. Kang, Y.-H. Kim, H.-Y. Jung, J.-H. Lee and I.-J. Lee. 2012. Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of *Paecilomyces formosus* LHL10. *BMC Microbiol.*, 12: 3.

Khan, A.L., M. Hamayun, Y.-H. Kim, S.-M. Kang, J.-H. Lee and I.-J. Lee. 2011. Gibberellins producing endophytic *Aspergillus fumigatus* sp. LH02 influenced endogenous phytohormonal levels, isoflavonoids production and plant growth in salinity stress. *Process Biochem.*, 46: 440-447.

Khan, A.L., M. Waqas, M. Hamayun, A. Al-Harrasi, A. Al-Rawahi and I.-J. Lee. 2013. Co-synergism of endophyte *Penicillium resedanum* LK6 with salicylic acid helped *Capsicum annuum* in biomass recovery and osmotic stress mitigation. *BMC Microbiol.*, 13: 51.

Kiani, M.Z., T. Sultan, A. Ali and Z.F. Rizvi. 2016. Application of acc-deaminase containing PGPR improves sunflower yield under natural salinity stress. *Pak. J. Bot.,* 48(1): 53-56.

Kjeldahl, J. 1883. Neue methode zur bestimmung des stickstoffs in organischen körpern. *Fresen. J. Anal. Chem.*, 22: 366-382.

Kuldau, G. and C. Bacon. 2008. Clavicipitaceous endophytes: their ability to enhance resistance of grasses to multiple stresses. *Biol. Control*, 46: 57-71.

Lamour, K.H., R. Stam, J. Jupe and E. Huitema. 2012. The oomycete broad-host-range pathogen *Phytophthora capsici*. *Mol. Plant Pathol*., 13: 329-337.

Lee, S.H., Y.E. Cho, S.-H. Park, K. Balaraju, J.W. Park, S.W. Lee and K. Park. 2013. An antibiotic fusaricidin: a cyclic depsipeptide from *Paenibacillus polymyxa* E681 induces systemic resistance against *Phytophthora* blight of red-pepper. *Phytoparasitica*, 41: 49-58.

McCloud, E.S. and I.T. Baldwin. 1997. Herbivory and caterpillar regurgitants amplify the wound-induced increases in jasmonic acid but not nicotine in *Nicotiana sylvestris*. *Planta*, 203: 430-435.

Pieterse, C., A. van Wees, J. Ton, J. Van Pelt and L. Van Loon. 2002. Signalling in rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. *Plant Biol.*, 4: 535-544.

Pozo, M.J. and C. Azcón-Aguilar. 2007. Unraveling mycorrhiza-induced resistance. *Curr. Opin. Plant Biol.*, 10: 393-398.

Ryals, J.A., U.H. Neuenschwander, M.G. Willits, A. Molina, H.-Y. Steiner and M.D. Hunt. 1996. Systemic acquired resistance. *Plant Cell*, 8: 1809.

Seskar, M., V. Shulaev and I. Raskin. 1998. Endogenous methyl salicylate in pathogen-inoculated tobacco plants. *Plant Physiol.*, 116: 387-392.

Singh, R., S.K. Soni, R.P. Patel and A. Kalra. 2013. Technology for improving essential oil yield of *Ocimum basilicum* L. (sweet basil) by application of bioinoculant colonized seeds under organic field conditions. *Ind. Crop Prod.*, 45: 335-342.

Smith, J.L., C.M. De Moraes and M.C. Mescher. 2009. Jasmonate-and salicylate-mediated plant defense responses to insect herbivores, pathogens and parasitic plants. *Pest Manag. Sci.*, 65: 497-503.

Sophearetha, M., S. Chan, K.W. Naing, Y.S. Lee, H.N. Hyun, Y.C. Kim and K.Y. Kim. 2013. Biocontrol of late blight (*Phytophthora capsici*) disease and growth promotion of pepper by *Burkholderia cepacia* MPC-7. *Plant Pathol. J.*, 29: 67.

Umashankari, J. and C. Sekar. 2011. Comparative evaluation of different bioformulations of PGPR cells on the enhancement of induced systemic resistance (ISR) in rice *P. oryzae* pathosystem under upland condition. *Curr. Bot.*, 2: 12-17.

Verma, J.P., J. Yadav, K.N. Tiwari and A. Kumar. 2013. Effect of indigenous *Mesorhizobium* spp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer arietinum* L.) under sustainable agriculture. *Ecol. Eng.*, 51: 282-286.

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