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Interactions between *Pseudomonas* spp. and their role in improving the red pepper plant growth under salinity stress

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ABSTRACT

Solitary inoculation of bacteria has been studied widely for plant growth development and amelioration of salinity stress but co-inoculation of bacteria for salt stress amelioration in red pepper plants has been less studied till date. Here, we investigated the co-inoculation effect of *Pseudomonas frederiksbergensis* OB139 and *Pseudomonas vancouverensis* OB155 in red pepper plant growth characteristics, plant photosynthesis pigments, ethylene emission, and antioxidant properties under 0, 50, 100 and 150 mM salt stress and compared them with non-inoculated control and single inoculation of each isolate. Results showed increasing concentrations of salinity stress arrested the normal plant growth, increased the stress ethylene levels, disrupted the photosynthetic parameters and also influenced the antioxidant enzymatic activities in non-inoculated control plants. Co-inoculation of 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing *Pseudomonas* spp. significantly reduced the stress ethylene emission and contributed to a significant increase in plant growth compared to single inoculation and non-inoculated control. Catalase activity which was significantly increased in co-inoculated red pepper plants compared to other treatments imply its ability to efficiently neutralize the hydrogen peroxide ions formed as a result of oxidative stress in plants under salinity stress. Besides, significant reduction in malondialdehyde (MDA) content can be correlated to the increased salt tolerance in co-inoculated red pepper plants. Lastly, the increased content of photosynthetic pigments suggest the importance of co-inoculation in improving photosynthesis of red pepper plants. Together, the data demonstrated the functional compatibility of the ACC deaminase producing bacterial isolates and their role in improving the plant physical and biochemical characteristics under salinity stress.

1. Introduction

Salinization of agricultural lands is a crucial hostility to crop yield and productivity. It has been estimated that around 1.5 billion ha of cultivated lands are affected by salinity (Selvakumar et al., 2014). Soil salinity is claiming about 3 ha of arable land from conventional crop farming every minute and it is expected that more than 50% of arable land would be affected by salinity by 2050 (Shrivastava and Kumar, 2015). On the other hand, it is estimated that Asia, Pacific and Australia accounts for the largest (30%) salt affected land used for agricultural practices (FAO, W.F.P., 2015; Sharma and Chaudhari, 2012). The salinity induced land degradation accounts for global crop production economic loss of US$ 27.3 billion (Qadir et al., 2014).

The detrimental effect on plant physiology under salinity stress results from the osmotic imbalance caused by the presence of excessive Na^+ and Cl^- ions in the soil (Munns and Tester, 2008; Tavakkoli et al., 2010; Yaish et al., 2016). The surplus presence of these ions cause imbalance in nutrient uptake and reduce availability of nutrients in soil (Moradi and Tahmourespour, 2011; Yaish and Kumar, 2015). The early symptoms observed in plants under salt stress includes necrosis of shoot and root, delay of leaf appearance, reduction in leaf surface area and internode lengths (Rajendran et al., 2009; Rahneshan et al., 2018). At the onset of salinity stress, root system of plants are primarily affected (Koyro, 2006) that leads to ionic imbalance in plants. It is followed by variety of consequences, which includes impairment of stomatal conductance leading to reduction in CO_2 assimilation in leaves thus affecting the photosynthetic efficiency of the plants (Chaves et al., 2009; Sarabi, 2017). Additionally, salinity induces reactive oxygen species (ROS) like hydrogen peroxide and malondialdehyde (MDA) which result in increased oxidative stress on plants (Chatterjee et al., 2017). The

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stress also induces ethylene levels, widely termed as "stress ethylene" (Heydarian et al., 2016) which is reported widely to exert adverse effects in plant physiology (Stearns and Glick, 2003; Ali et al., 2014).

The deleterious effect of salinity can be alleviated by the inoculation of plant growth promoting bacteria (PGPB) which has been widely accepted as a cost effective and sustainable procedure for salt stress amelioration (Jha and Subramanian, 2014). PGPB can stimulate plant growth and alleviate salinity stress through a range of mechanisms which include colonization of rhizopshere (Subramanian et al., 2015b), production of phytohormones and 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Siddikee et al., 2015), fixation of atmospheric nitrogen (Islam et al., 2013) and synthesis of ROS scavenging enzymes (Nautiyal et al., 2013; Sarkar et al., 2018). Previous studies mentioned about the role of *Pseudomonas* sp. in plant growth promotion and in regulating nutrient accumulation, and stress responses in rhizosphere (Lugtenberg and Dekkers, 1999). In addition, pseudomonads group of bacteria have been reported to colonize plant rhizosphere, making them potential candidates for the development of bio-incoulant (Dekkers et al., 2000).

Most of the approaches for plant growth promoting inoculants are relied on the application of single microbe which might account for the inconsistencies in field conditions (Gadhave et al., 2016; Perkins and Bennett, 2017). The recent development of designing microbial consortia which contains more than one microbe has led to enhanced level of plant growth and stress alleviation (Felici et al., 2008; Krishnamoorthy et al., 2016). Each microbe present in such a consortium are capable of competing with native rhizospheric establishments and also complement functionally for plant growth promotion (Welbaum et al., 2004). Thus, this work aimed to investigate the effect of co-inoculation of OB139 (*Pseudomonas frederiksbergensis*) and OB155 (*Pseudomonas va*) on ameliorating salinity stress and compare them with single inoculation or non-inoculated control. It was hypothesized that co-inoculation would augment the salinity tolerance in plants with improved ACC deaminase activity, reduction of stress ethylene, and enhancement of antioxidant and photosynthetic parameters. This hypothesis was tested on red pepper plants (*Capsicum annum* L.) which is cultivated in more than 3 million ha of land around the globe and is a very common ingredient in Korean cuisine (Kurunc et al., 2014; Patra et al., 2016).

2. Materials and methods

2.1. Experimental design

In the present study, different concentrations of sodium chloride (NaCl) were applied in the pots containing soil which were either inoculated (*Pseudomonas frederiksbergensis* OB139, *Pseudomonas vancouverensis* OB155 and co-inoculated with both) or non-inoculated and following parameters were examined : (1) how bacterial inoculation improves the plant physiological parameters (root length, shoot length and dry weight); (2) how plant ethylene emissions respond to individual effects of NaCl and individual and co bacterial inoculation; (3) how salt stress and co-inoculation of bacteria affects the photosynthetic characteristics, antioxidant enzyme activities and lipid peroxidation.

2.2. Bacterial strains, inoculum preparation and plant growth promoting characteristics

Pseudomonas frederiksbergensis OB139 (GenBank Accession no. KF424301) and *Pseudomonas vancouverensis* OB155 (GenBank Accession no. KF424309) were used in this study which were previously isolated from experimental fields of Chungbuk Agricultural Research and Extension Services, South Korea (Subramanian et al., 2016). Inoculum was prepared by growing the bacterial isolates OB139 and OB155 in nutrient broth medium at 30 °C for 24 h followed by centrifugation (5000 rpm for 5 min at 4 °C), washing and re-suspension in 30 mM $MgSO_4$ to obtain a cell density of 10^8 cfu ml^{-1} ($OD_{600} = 0.8$). The treatments included: i) non-inoculated control ii) inoculation with OB155 iii) inoculation with OB139 iv) co-inoculation with OB155 and OB139. For co-inoculation, equal amounts of two bacterial cultures were mixed and used for experiment. The plant growth promoting characteristics of the isolates taken singly and together were studied at normal conditions as described by Subramanian et al. (2016).

2.3. Plant materials, seed bacterization and greenhouse experiment

Red pepper (*Capsicum annuum* L. cv. Bulmat) seeds (Syngenta seeds, Seoul, Republic of Korea) were used in this study. Seeds were surface sterilized using 70% ethanol for 30 s and 2% sodium hypochlorite for 1 min, followed by several washes with sterile distilled water. The bacterization was performed by soaking the surface sterilized seeds in bacterial suspension prepared as mentioned above for 4 h whereas the control seeds were soaked only in $MgSO_4$. Red pepper seeds were sown in seedling trays with 40 g of nursery soil (Nongwoo-Bio Co., Ltd., Yeoju-gun, Gyeonggi-do, Republic of Korea) in each hole. Initially, seeds were grown in the dark condition to accomplish the germination process inside the growth chamber (DS 54 GLP, DASOL Scientific Co., Ltd., Korea) and the below mentioned conditions were maintained till germination: light intensity of 18 $\mu mol\, m^{-2}\, s^{-1}$ for 12-h photoperiod; a constant day and night temperature of 25 °C; and relative humidity of 70%. After seven days seedlings were transferred to plastic pots (pot height × pot diameter (top) × pot diameter (base): 16 × 16 × 10 cm) having 500 g of soil in each pot and they were grown under normal greenhouse conditions. 10 ml of bacterial suspension (prepared as mentioned above) was added 14 days after sowing (DAS) near the root zone of each seedling. Six replicates were used for each treatment.

2.4. Salt stress application

Three levels of salt stress, 50 mM, 100 mM and 150 mM NaCl were imposed. To avoid osmotic shock, 25 mM of salt solution was imposed gradually by applying sodium chloride solution to each pot on alternative days and desired concentration of 50, 100 and 150 mM were achieved after 2, 6 and 10 days respectively. Control plants were well watered throughout the experiment period. The water leaching from the pot was controlled by keeping the soil water level less than the water holding capacity. The data on soil electrical conductivity (EC) recorded at the time of harvest are provided in supplementary File S1. 50 DAS plants were harvested and the plant growth characteristics such as root length, shoot length were measured. Besides, number of leaves was also recorded. The total dry biomass of the plants were measured after drying the plants at 70 ℃ in an oven for 72 h.

2.5. Estimation of ethylene emission from red pepper seedlings and red pepper leaves

In a separate experiment, ethylene emission from red pepper seedlings was measured according to Mayak et al. (2004). Briefly, surface sterilization and seed bacterization were performed as mentioned earlier. Nextly, 30 red pepper seeds were either imbibed in bacterial suspension or 30 mM $MgSO_4$ for a period of 2 h. Following that, the liquid was drained, and seeds were placed inside a 120 ml narrow neck bottle containing a filter paper soaked with 2 ml of sterile distilled water and incubated in plant growth chamber under conditions identical to the main experiment. Three to four days after seedling germination, the excess liquid was drained and 2 ml of 0, 50 or 100 mM of NaCl solution was added. 4 h after the treatment, the bottles were closed for 2 h with a rubber septum and 1 ml of gas was sampled from the headspace and analyzed with a gas chromatograph with Poropak-Q column (dsCHROM 6200, Donam Instruments Inc., Republic of Korea). Six replicates were used for each treatment.

2.6. Estimation of plant antioxidant enzyme activities and photosynthetic parameters

Antioxidant enzyme activities were determined from red pepper leaves obtained from plants harvested 50 DAS. The fresh leaf samples were ground in liquid nitrogen and stored at –80 °C. Homogenization of ground leaf sample (0.5 g) was performed in 10 ml of 50 mM of potassium phosphate buffer, 1% (w/v) polyvinylpyrrolidone (pH 7.8) and the sample was incubated at 40 °C for 10 min. The homogenized sample was filtered and centrifuged (VS–24SMTi, High Speed Refrigerated Centrifuge, Vision Scientific Co. Ltd., Korea) at 4000 × g for 15 min at 4°C. The supernatant was collected and used for estimation of enzyme activities. The catalase (CAT) activity was estimated by a hydrogen peroxide assay where 0.2 ml of enzyme extract was taken in 1 ml reaction mixture (containing 65 mM hydrogen peroxide in 60 mM sodium phosphate buffer) and incubated at room temperature for 4 min. The reaction was stopped by using 1 ml 32.4 mM of ammonium molybdate and yellow complex was measured at 405 nm (Goth, 1991). Superoxide dismutase (SOD) activity was measured from the enzyme extract after adding the assay mixture (50 mM phosphate buffer: pH 7.8, 9.9 mM L-methionine, 57 mM NBT, 0.025% w/v Triton X-100, 0.0044% w/v riboflavin) into it. Following reaction, the activity was recorded according to the photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm (Ding et al., 2011). The ascorbate peroxidase (APX) activity was determined by the oxidation of ascorbic acid to dehydroascorbate. The enzyme extract was added in to the reaction mixture (50 mM potassium phosphate buffer: pH 7.0, 0.3 mM ascorbic acid, 0.1 mM H_2O_2: hydrogen peroxide, 0.1 mM EDTA). The activity was calculated by the means of extinction coefficient 2.8 mM^{-1} cm^{-1} at 290 nm (Ding et al., 2011). Hydrogen peroxide content was measured as described by Theocharis et al. (2012). Lipid peroxidation was measured by quantifying the amount of MDA formation following the protocol as described by Heath and Packer (1968). Briefly, the fresh leaves were grounded using ice cold phosphate buffer solution. Following centrifugation, 1 ml of supernatant was added to reaction mixture containing 4 ml of 0.5% (w/v) thiobarbituric acid (TBA) solution which consisted of 20% TBA. The mixture was heated at 95 °C for 30 min. The reaction was terminated by keeping the reaction mixture in a cold water bath. Finally absorbance of the mixture was recorded at 532 and 600 nm. The MDA content was calculated subtracting A_{532} from A_{600} and multiplied by the extinction coefficient 155 mm^{-1} cm^{-1}. Chlorophyll *a*, chlorophyll b and carotenoid content were determined from the harvested red pepper leaves. Pigments related to photosynthesis were extracted from fresh leaf samples using 80% acetone and quantification was done following the protocol as mentioned by Porra et al. (1989).

2.7. Statistical analysis

A randomized block design was maintained and the data from experimental results were subjected to a two-way analysis of variance (ANOVA). A schematic diagram of the experimental setup is provided in supplementary File S2. The significant differences between the means were determined by Tukey's test at $P < 0.05$ using SAS package, Version 9.4.

3. Results

3.1. Plant growth promoting characteristics of the bacterial isolates

The ACC deaminase activity and other plant growth promoting characteristics were much improved in co-culture of bacterial isolates compared to single bacterial isolate (Table 1).

3.2. Ethylene emission from red pepper seedling in response to salinity stress and bacterial inoculation

Ethylene emissions were measured in a separate experiment in red pepper seedlings. These measurements indicated with increase in salinity levels ethylene emission rates also increased in non-inoculated control plants (Fig. 1). Co-inoculation of bacteria significantly reduced the ethylene emission at all levels of salinity stress compared to non-inoculated control and solitary application of bacteria. At 100 and 150 mM of salinity stress, though single bacterial inoculation also significantly reduced the ethylene emission compared to non-inoculated control but co-inoculation resulted in a much higher rate of decrease compared to other treatments.

3.3. Effects of salinity stress and bacterial inoculation on plant growth characteristics

The plant growth characteristics mainly the shoot length, root length, dry bio mass and number of leaves per plant were recorded to investigate the effect of individual and co-inoculation of OB155 and OB139 on red pepper plant growth in response to salinity stress (Fig. 2). Root length decreased with increasing salinity levels in non-inoculated control (Fig. 2A). Bacterial inoculation improved the root length significantly compared to control in all levels of salinity stress. However, at 100 and 150 mM of salt stress, co-inoculation significantly increased the root length compared to non-inoculated control and single bacterial inoculation. Co-inoculation increased the root length compared to control by 31.2% in 50 mM, 72.9% in 100 mM, 65.1% in 150 mM of salt stress. With increase in salt stress shoot length was also reduced in non-inoculated control plants (Fig. 2B). Co-inoculation significantly increased the shoot length in all levels of salinity stress compared to other treatments. Shoot length was increased by 16% in 50 mM, 21.3% in 100 mM and 23.3% in 150 mM salt stress in co-inoculated plants compared to control. Dry mass of red pepper plant decreased with the increasing salt concentration in non-inoculated control plants (Fig. 2C). Co-inoculation significantly improved the dry weight of the plants in all levels of salinity stress and increased the dry mass by 36.2%, 61.5% and 64.5% at 50, 100 and 150 mM of salt stress respectively compared to control (Fig. 2C). After recording the number of leaves in plants, it was observed that co-inoculation significantly increased the number of leaves in all levels of salinity stress which might have contributed to the increased biomass (Fig. 2D).

3.4. The effects of salt stress and inoculation of bacteria on plant antioxidant enzyme activities

APX activity increased with increasing levels of salinity stress in non-inoculated control (Fig. 3A). Single and co-inoculation significantly reduced the APX activity in 50 and 150 mM of salinity stress compared to control but decrease in APX activity was much pronounced upon co-inoculation in all levels of salinity stress compared to other treatments. Co-inoculation decreased the APX activity by 41%, 21.5% and 20.8% at 50, 100 and 150 mM of salt stress respectively compared to control.

SOD activity followed a similar trend as APX in red pepper plants under salinity stress (Fig. 3B). Co-inoculation significantly decreased the SOD activity at all levels of salinity stress compared to all treatments and was reduced by 18.3%, 30.8% and 21.65% at 50, 100 and 150 mM of salt stress respectively compared to control.

CAT activity decreased with the increasing salt concentration in non-inoculated control plants (Fig. 3C). Co-inoculation effect of bacteria on CAT activity was evident at higher levels of stress (100 and 150 mM) where activity of the enzyme was significantly increased compared to all treatments.

Co-inoculation significantly reduced the total H_2O_2 production in all levels of salinity stress compared to other studied treatments (Fig. 3D). At 150 mM, single inoculation with OB155 and OB139 decreased the

Table 1
Plant growth promoting characteristics of the isolates studied under normal conditions.

Characteristics	OB155	OB139	OB155 + OB139
ACC deaminase (nmol α-KB mg^{-1} protein h^{-1})	33.37 ± 0.96	24.62 ± 1.63	43.37 ± 1.27
IAA (μg ml^{-1})	7.61 ± 0.84 (With trp) 4.43 ± 0.55 (without trp)	10.64 ± 0.92 (With trp) 6.42 ± 1.63 (without trp)	14.84 ± 0.95 (With trp) 6.21 ± 0.67 (without trp)
Siderophore production	+	–	+
Salicylic acid production (mg l^{-1})	1.53 ± 0.34	0.94 ± 0.34	3.04 ± 0.42
Phosphate solubilization	+	+	+

Values in each column are the means of three replications ± standard error (SE). Pseudomonas vancouverensis OB155, Pseudomonas frederiksbergensis OB139, ACC- 1-aminocyclopropane-1-carboxylate, IAA- Indole acetic acid, trp- tryptophan 500 μg ml-1.

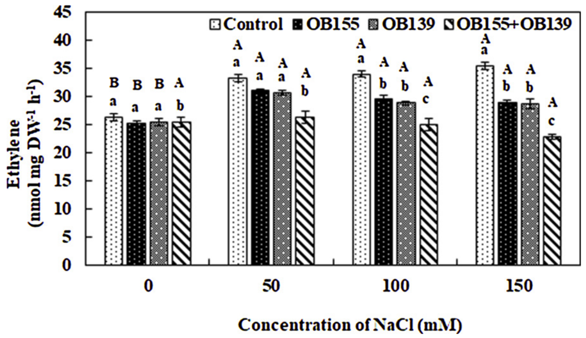

Fig. 1. Effect of single inoculation (*Pseudomonas vancouverensis* OB155, *Pseudomonas frederiksbergensis* OB139*)* and co-inoculation (OB155 + OB139) on ethylene emission from red pepper seedlings studied under different levels of salinity stress. Different letters indicate significant differences $P < 0.05$ within the treatments at each salt levels (a, b, c) or among salt levels for each treatment (A, B) by Tukey's test. Each value represents the mean of six replicates ± standard error (SE).

H_2O_2 content by 4.8% and 3% respectively compared to control, whereas co-inoculation of OB155 and OB139 reduced the H_2O_2 concentration by 27% in red pepper plants.

3.5. The effects of salt stress and inoculation of bacteria on photosynthetic parameters and lipid peroxidation

Foliar chlorophyll *a*, b and carotenoid content decreased upon salinity stress in non-inoculated control plants (Fig. 4). Single inoculation of bacteria helped red pepper plants to increase the chlorophyll *a* and *b* content at 150 mM and 100, 150 mM of salt stress respectively compared to control. However, co-inoculation effect was more prominent as it increased the chlorophyll content significantly at 150 mM of salinity stress compared to all treatment studied (Fig. 4 A, B).

Carotenoid content which is considered important for electron transport and photosynthesis decreased with severity of salt stress in non-inoculated control plants (Fig. 4C). Co-inoculation proved to be most effective as it increased the carotenoid content in all levels of salinity stress. At 50 and 150 mM of salt stress, co-inoculation worked efficiently as it showed significant increase in carotenoid content compared to all treatments studied.

The extent of membrane damage in response to stress was determined by the non-enzymatic oxidation of fatty acids, where

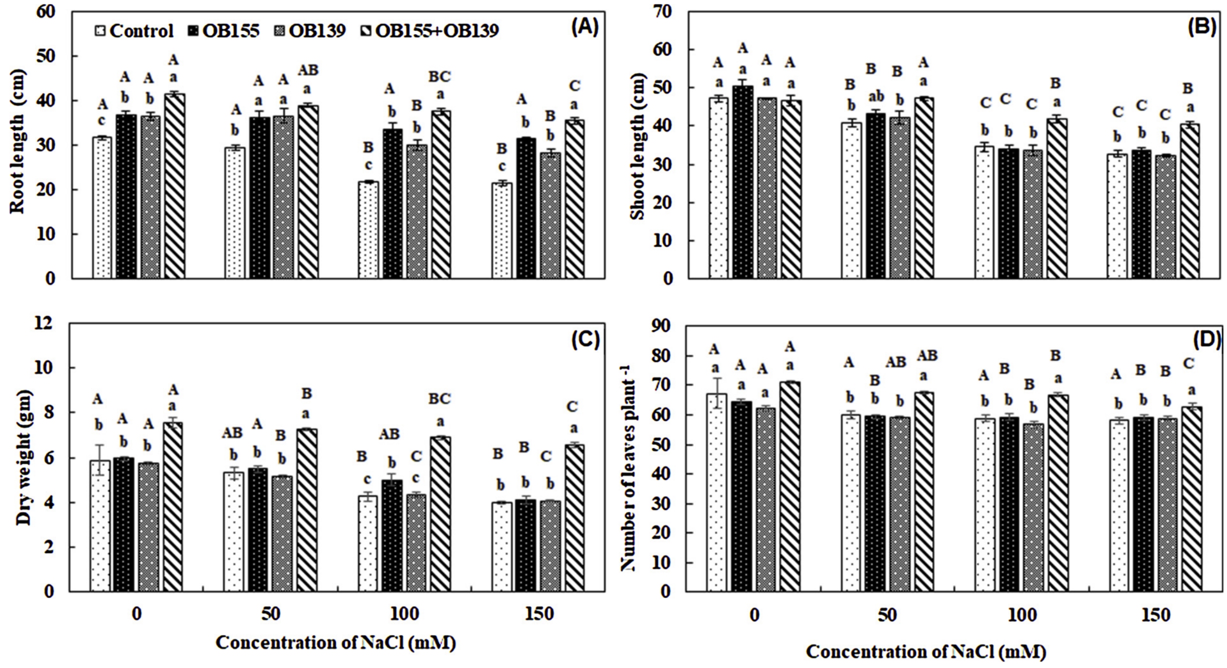

Fig. 2. Effect of single inoculation (*Pseudomonas vancouverensis* OB155, *Pseudomonas frederiksbergensis* OB139*)* and co-inoculation (OB155 + OB139) on plant growth promoting characteristics of red pepper plants studied under different levels of salinity stress. (A) Root length (B) Shoot length (C) Dry weight (D) Number of leaves. Different letters indicate significant differences $P < 0.05$ within the treatments at each salt levels (a, b, c) or among salt levels for each treatment (A, B, C) by Tukey's test. Each value represents the mean of six replicates ± standard error (SE).

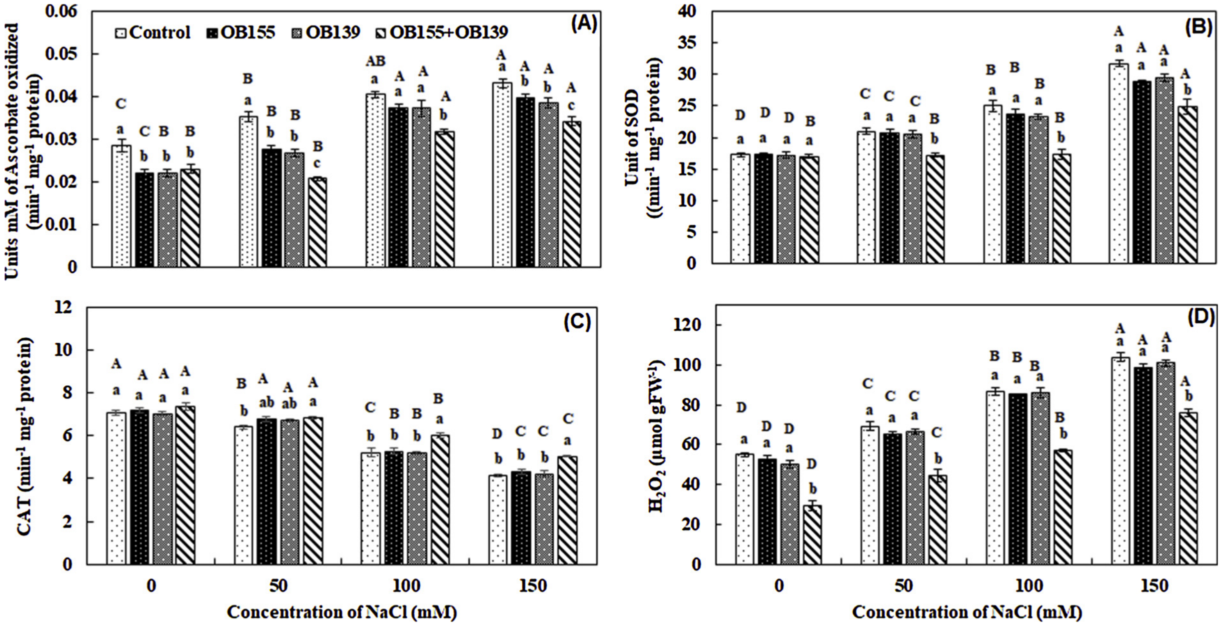

Fig. 3. Effect of single inoculation (*Pseudomonas vancouverensis* OB155, *Pseudomonas frederiksbergensis* OB139*)* and co-inoculation (OB155 + OB139) on hydrogen peroxide content and antioxidant enzyme activities of red pepper plants studied under different levels of salinity stress. (A) Ascorbate Peroxidase (APX) (B) Superoxide dismutase (SOD) (C) Catalase (CAT) (D) Hydrogen peroxide content (H_2O_2). Different letters indicate significant differences $P < 0.05$ within the treatments at each salt levels (a, b, c) or among salt levels for each treatment (A, B, C, D) by Tukey's test. Each value represents the mean of six replicates ± standard error (SE).

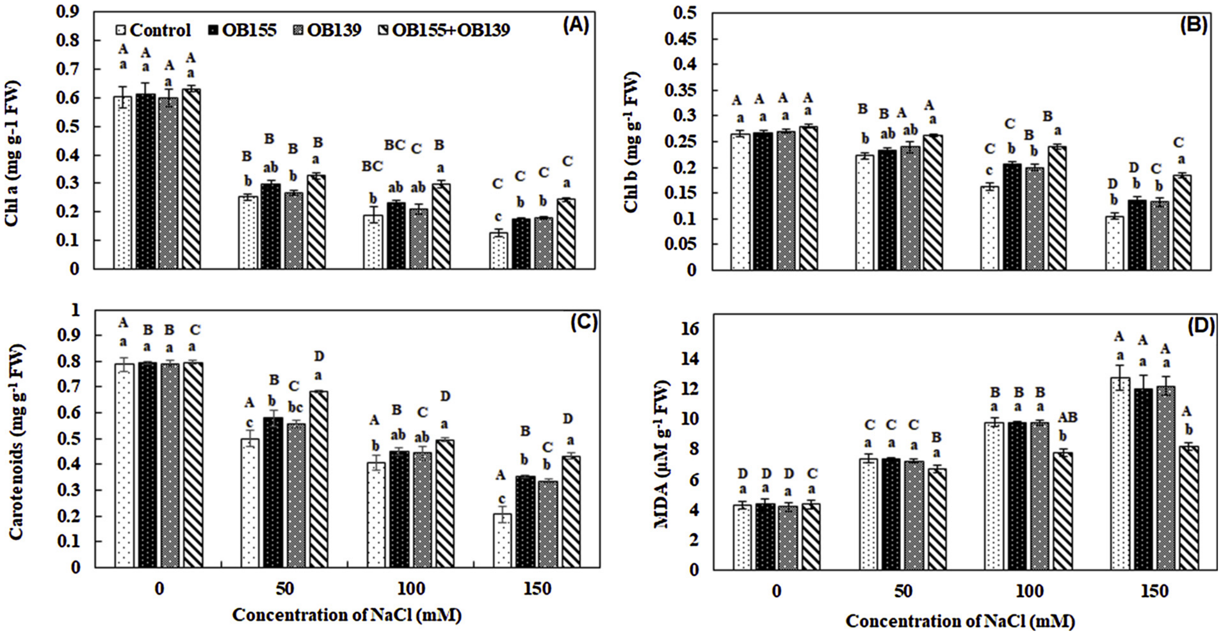

Fig. 4. Effect of single inoculation (*Pseudomonas vancouverensis* OB155, *Pseudomonas frederiksbergensis* OB139*)* and co-inoculation (OB155 + OB139) on photosynthetic characteristics and malondialdehyde (MDA) content of red pepper plants studied under different levels of salinity stress. (A) Chlorophyll *a* (B) Chlorophyll b (C) Carotenoid content (D) MDA content. Different letters indicate significant differences $P < 0.05$ within the treatments at each salt levels (a, b, c) or among salt levels for each treatment (A, B, C, D) by Tukey's test. Each value represents the mean of six replicates ± standard error (SE).

generation of MDA is considered as a marker of lipid peroxidation (Fig. 4D). Non-inoculated red pepper plants under non-stressed conditions showed increase in MDA content with increasing salinity stress. At 100 and 150 mM of salt stress co-inoculation treatment decreased the MDA content significantly compared to other treatments. Co-inoculation reduced the MDA content by 9.3%, 20.4% and 35.5% at 50, 100 and 150 mM of salt stress respectively compared to control.

4. Discussion

The current study evaluated the ability of co-inoculated bacterial strains to alleviate salinity stress in red pepper plants. The observed results demonstrated co-inoculation significantly enhanced the red pepper plant growth under different salinity levels compared to the other treatments. The pronounced effects by co-inoculation were imparted to the salt affected red pepper plants by moderating the antioxidant enzyme activities, photosynthetic activities and stress ethylene levels.

Ethylene is considered as a stress marker under abiotic and biotic stresses (Stearns and Glick, 2003). Adverse effects of ethylene on plant growth has been studied widely (Belimov et al., 2001; Saravanakumar and Samiyappan, 2007). Osmotic imbalance after salt amendment activates the ACC gene cluster in plants (Skirycz et al., 2011), those are responsible for the activation of ethylene responsive genes. To withstand these negative effects, microbes possessing the ACC deaminase activity has been reported to work efficiently as they can cleave ACC to its byproducts thus reducing ethylene levels in plants (Glick, 2005; Sun et al., 2009; Rashid et al., 2012). In the present study, degree of reduction in stress ethylene levels (Fig. 1) under salinity stress was much pronounced upon co-inoculation compared to the single bacterial inoculation implying the improved ACC deaminase activity (Table 1) being imparted by the bacterial consortia.

Red pepper being a salt sensitive plant (Navarro et al., 2002; Aktas et al., 2006) cannot regulate the uptake of sodium ions efficiently, paralyzing the plant mechanisms to carry out the biological processes, leading to reduction in plant biomass (Munns et al., 2006; Bashan et al., 2014). In the current study, salinity stress decreased the overall red pepper plant growth in non-inoculated control plants (Fig. 2). The reduction in red pepper growth after salt amendment is in line with Tuna et al. (2007) where tomato plant growth was highly affected by salinity stress. On the other hand, single inoculation and co-inoculation of bacterial isolates significantly improved the red pepper plant growth and improved the dry biomass under different levels salinity stress. Several studies have demonstrated the ability of PGPB to stimulate the growth of variety of crops which include canola, lettuce, tomato, apple, citrus and beans (Lemanceau, 1992; Kloepper, 1994; Hall et al., 1996). Comparing the degree of improvement, co-inoculation increased the plant growth significantly compared to single inoculation. The improved ACC deaminase and indole acetic acid (IAA) activity of the bacterial consortia (Table 1) might have worked in a concerted network and helped plant to withstand the adverse effects of salinity stress. IAA produced by bacteria is reported to enhance the flexibility of plant cell wall and increase the release of saccharides which help the microbes to efficiently colonize the plant roots. (Etesami et al., 2015). Besides, plant require several nutrients for their growth, iron is one of them which is generally unavailable to plants as it is insoluble in soil solutions (Vessey, 2003). Kloepper et al. (1980) proposed PGPB producing siderophores can effectively bind iron and make it available for plants thus contributing to plant growth. In the current study, bacterial isolates when co-inoculated have complemented each other functionally as one of them possess siderophore and other don't which might have contributed to improved plant growth in the co-inoculated plants (Table 1). Bano and Fatima (2009) showed the promising effect of co-inoculating *Rhizobium* and *Pseudomonas* species on physiological properties of salt affected maize plants which strongly support the observations of the current study. Additionally, co-inoculation of *Methylobacterium* sp. with nitrogen fixing and phosphate solubilizing bacterial isolates were shown to improve plant physiological properties in various crop plants (Madhaiyan et al., 2010) Besides, in a recent study conducted on soybean plants co-inoculation of endophytic bacteria with *Bradyrhizobium* species were seen to improve the plant growth and nutrient uptake compared to their solitary inoculation (Subramanian et al., 2015a).

Salinity stress leads to oxidative damage in plants which produces mainly ROS molecules namely hydroxyl ion, singlet oxygen and hydrogen peroxide that damages cell membranes thus harming the plant cell structures (Apel and Hirt, 2004). In order to withstand the oxidative stress, the plants have developed a plethora of biochemical and physiological mechanisms which is made of several enzymes which can scavenge the ROS species. The enzymes APX, SOD, CAT and redox ions known as prominent antioxidant enzymes work in a network to perform the detoxification of ROS species (Abbas et al., 2013; AbdElgawad et al., 2016). In the present study, the inoculated red pepper plants demonstrated improved ROS scavenging enzymatic activities compared to non-inoculated control plants (Fig. 3). Interestingly, the enzyme activities varied according to inoculation pattern. The results showed co-inoculated plants to exhibit improved enzyme activities compared to plants inoculated with a single bacteria. The co-inoculated plants showed a significant decrease in SOD activity with the increasing salinity levels compared to single inoculated plants and control implying nullification of ROS molecules thus leading to salinity stress amelioration (Alscher et al., 2002; Cavalcanti et al., 2004). APX activity also decreased alike SOD, but at higher salinity levels effect of co-inoculation was observed to be more profound compared to single inoculation (Jebara et al., 2005). The higher and significant reduction in the antioxidant enzyme activities in co-inoculated plants can be attributed to the decrease in production of hydrogen peroxide molecules, where the co-inoculation of bacteria controlled the changes in ion concentration in plants affected by external perturbations in soil (Gururani et al., 2013). The CAT activity was significantly augmented in co-inoculated plants under high salinity stress hinting their effective adaptation to salinity stress by eradicating the ROS species (Kohler et al., 2008; Subramanian et al., 2015b). MDA content (Fig. 4D) considered as a marker to estimate the extent of membrane damage due to oxidative stress were significantly reduced in co-inoculated plants cueing with some earlier reports where enhanced stress tolerance in plants was related to reduced lipid peroxidation (Bharti et al., 2014).

Osmotic imbalance in plants results in dehydration of plant cells which lead to disturbances in stomatal opening and photosynthesis, finally affecting the plant growth (Chatterjee et al., 2018). The increase in photosynthetic pigments (Fig. 4) as observed by the significant increase in chlorophyll and carotenoid content in co-inoculated plants suggest the ability of bacterial co-inoculation to nullify the adverse effects of salt stress by enhancing the activity of electron transporters related to photosynthesis (Young and Britton, 1990; Pinnola et al., 2016). Furthermore, the increase in number of leaves (Fig. 2D) upon co-inoculation can be also related to the improved photosynthetic activity in co-inoculated plants as salinity stress is reported to affect the growth of new leaves as they do not get sufficient carbohydrates for growth and metabolism (Munns, 1993).

5. Conclusions

Taking together the results of the present study demonstrated co-inoculation of bacterial isolates OB139 (*Pseudomonas frederiksbergensis*) and OB155 (*Pseudomonas vancouverensis*) improved the plant physiological properties under salinity stress compared to single inoculation or non-inoculated control by improving the antioxidant enzyme activities, increasing the content of photosynthetic pigments and modulating the stress ethylene levels. The results prove the compatibility of the isolates in the rhizosphere and also support the concept of functional complementation which exerted beneficial effects on plant growth and also alleviated salinity stress. The strains used has already been reported to alleviate chilling stress effects when inoculated solitarily in tomato plants (Subramanian et al., 2015b). The results from the current study for alleviation of salinity stress will add an extra attribute to it and can be exploited further for unravelling the mechanistic approaches for the development of a microbial consortia under abiotic stresses.

Conflict of interest

None.

Author contributions

SS, PC and TS: design of the research; PC, SS: Performance of the research; SS, PC: Data analysis; SS, PC and TS: Data Interpretation; SS, PC, ARC, SA: writing the manuscript, TS: critical revision of the manuscript

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Appendix A. Supplementary data

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