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Regulation of Ethylene Biosynthesis Under Salt Stress in Red Pepper (*Capsicum annuum* L.) by 1-Aminocyclopropane-1-Carboxylic Acid (ACC) Deaminase-producing Halotolerant Bacteria

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Abstract The present study was carried out to understand the mechanism of salt stress amelioration in red pepper plants by inoculation of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase-producing halotolerant bacteria. In general, ethylene production, ACC concentration, ACC synthase (ACS), and ACC oxidase (ACO) enzyme activities increased with increasing levels of salt stress. Treatment with halotolerant bacteria reduced ethylene production by 47–64%, ACC concentration by 47–55% and ACO activity by 18–19% in salt-stressed (150 mmol NaCl) red pepper seedlings compared to uninoculated controls. ACS activity was lower in red pepper seedlings treated with *Bacillus aryabhattai* RS341 but higher in seedlings treated with *Brevibacterium epidermidis* RS15 (44%) and *Micrococcus yunnanensis* RS222 (23%) under salt-stressed conditions as compared to uninoculated controls. A significant increase was recorded in red pepper plant growth under salt stress when treated with ACC deaminase-producing halotolerant bacteria as compared to uninoculated controls. The results of this study collectively suggest that salt stress enhanced ethylene production by increasing enzyme activities of the ethylene biosynthetic pathway. Inoculation with ACC deaminase-producing halotolerant bacteria plays an important role in ethylene metabolism, particularly by reducing the ACC concentration, although a direct effect on reducing ACO activity was also observed. It is suggested that growth promotion in inoculated red pepper plants under inhibitory levels of salt stress is due to ACC deaminase activity present in the halotolerant bacteria.

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Introduction

Ethylene is a gaseous hormone and is involved in a wide range of biological processes (Abeles and others 1992; Bleecker and Kende 2000). However, a variety of stresses, such as flooding, wounding, mechanical damage, damage by disease, water deficit/drought, air pollution, nutrient deficiency, mineral toxicity, and salinity, are known to increase ethylene production, and increased levels of ethylene aggravate the stress effect (Abeles and others 1992; Mattoo and Suttle 1991; Mayak and others 2004; Morgan and Drew 1997; Wang and others 1990). In particular, an increase in the ethylene level inhibits plant growth and induces senescence irrespective of growth and developmental stages which leads to premature death (Abeles and others 1992; Feng and Barker 1992; Helmy and others 1994; Mattoo and Suttle 1991; Mayak and others 2004).

Ethylene production in plants is regulated by the mitogen-activated protein kinase (MAPKs) cascade and is activated in response to diverse external stimuli (Hahn and March 2009), that is, salt stress activates the MAPK cascade and induces ethylene biosynthesis in plants. The steps involved in ethylene biosynthesis in higher plants start with the conversion of methionine to *S*-adenosyl-methionine (SAM), which is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) and, in turn, ACC is converted to ethylene by ACC oxidase (ACO). The enzymes ACS and ACO are encoded by a multigene family and each gene is regulated independently by specific stresses (Barry and others 1996; Bleecker and Kende 2000; Ge and others 2000). ACS is considered the

rate-limiting step in ethylene biosynthesis (Chae and others 2003; Wang and others 2002) and a stress condition might activate the MAPK cascade and cause dramatic increases in ACS activity, ACC level, followed by ACO activity. Moreover, stress-level ethylene itself in plants is associated with the induction of ACS and ACO enzyme activity (Bleecker and Kende 2000; Wang and others 2002). Experimentally, evidence suggests that stress-induced (biotic or abiotic) ethylene production can be reduced by ACC deaminase-producing plant-growth-promoting rhizobacteria (PGPR) (Cheng and others 2007; Glick 2004; Glick and others 1998; Mayak and others 2004). In fact, higher plants release about 40% photosynthate through root exudates (Lynch and Whipps 1991), and it was postulated that a large portion of ACC produced under stress conditions may be exuded from plant roots (Bayliss and others 1997), which is then taken up by ACC deaminase-producing bacteria and hydrolyzed into ammonia and α-ketobutyrate (Glick and others 1998; Penrose and Glick 2001). Thus, the more ACC utilized by bacteria as an N source results in reduced ACC concentrations available for ACC oxidase to convert into ethylene. Thus, plant growth promotion under stress may be due to the presence of ACC deaminase activity in PGPR. It is now clear that the effect of stress and ACC deaminase-producing PGPR play an important role in ethylene biosynthesis and plant growth.

Some ACC deaminase-producing salt-tolerant bacteria have been reported to be effective in reducing ethylene biosynthesis and increasing plant growth promotion under salt stress (Cheng and others 2007; Mayak and others 2004), but the effect of salt stress and the involvement of ACC deaminase-producing halotolerant bacteria inoculation on ethylene metabolism and plant growth promotion is not well understood. Therefore, we investigated the effect of salt stress and ACC deaminase-producing halotolerant bacteria inoculation on ethylene production, metabolism, and growth promotion of red pepper plants.

Materials and Methods

Bacterial Strains and Growth Conditions

ACC deaminase-producing halotolerant bacteria *Bacillus aryabhattai* RS341, *Brevibacterium epidermidis* RS15, and *Micrococcus yunnanensis* RS222 were procured from our previous study. Except for *B. aryabhattai* RS341, the other two strains (*Br. epidermidis* RS15 and *M. yunnanensis* RS222) are able to produce IAA (Siddikee and others 2010). Bacterial strains were maintained on Tryptic soy agar (TSA) slants modified with 5% NaCl (pH 7.2) at 4°C in a refrigerator with trimonthly transfers.

Effect of Different Levels of Salt Stress on Ethylene Production of Red Pepper Seedlings

Ethylene emissions from red pepper seedlings were measured following the protocol of Madhaiyan and others (2007, 2006) with modification. Red pepper seeds (*Capsicum annuum* L.; Hungnong seeds, Seminis Korea Inc., Republic of Korea) were surface sterilized using 2% NaOCl for 1 min and with 70% ethanol for 30 s followed by thorough rinsing with sterile distilled water (five times). The seeds were sown in seedling trays (40 holes $tray^{-1}$ and 1 seed $hole^{-1}$) containing 150 g Biosangto-Mix bed soil (Heung Nong Co., Ltd, Incheon, Gyeonggi-do, Republic of Korea; containing 65–70% cocoa peat, 15–20% peat moss, 8–10% perlite and macronutrient (mg l^{-1}) NH_4–N, 80–100; NO_3–N, 150–200; available P_2O_5, 230–330; K_2O, 80–120; pH 5.5–6.5; moisture content 50–60%; and water holding capacity 35–40%) and incubated in a growth chamber (DS 54 GLP, DASOL Scientific Co., Ltd., Korea) at 25°C with a 12-h day–night photoperiod (relative humidity [RH] = 70% and light intensity = 18 μmol m^{-2} s^{-1}). Seven-day-old seedlings were exposed to different levels of salt stress (100, 150, and 200 mmol NaCl) for 7 days. Seeds and seedlings not treated with salt were used as negative controls and seedlings treated with salt were positive controls. Positive and negative controls were described earlier (Siddikee and others 2010). Red pepper seedlings (24 seedlings for each treatment) were uprooted and washed using respective concentration of saline water to remove soil from roots and were placed inside 120-ml NarrowNeck Mackarteny bottles. The bottles were kept open for 30 min to let air escape and then sealed for 4 h using a rubber septum. A 1-ml sample of the headspace air of each bottle was injected into a gas chromatograph (dsCHROM 6200, Donam Instruments Inc., Republic of Korea) packed with a Poropak-Q column at 70°C and equipped with a flame ionization detector. The amount of ethylene emission was expressed as pmol ethylene g^{-1} fresh weight h^{-1} by comparing the standard curve generated with pure ethylene (Praxair, Praxair Korea Co., Ltd.). Three replicates were used for each treatment. At the same time, 2–3 g of seedlings (root) from each treatment was weighed and stored at −80°C and subsequently used to assay the levels of ACC concentration and endogenous hydrolytic enzyme activities.

Measurement of ACC Level in Plant Tissue

ACC concentrations in plant tissue were measured following the protocol of Madhaiyan and others (2007). One gram of root sample was immediately frozen in liquid nitrogen and ground. ACC from frozen ground tissue was extracted using 5 ml 80% methanol containing butylated hydroxytoluene (BHT, 2 mg l^{-1}) as an antioxidant and

incubated at room temperature for 45 min. Samples were centrifuged at 2,000×*g* at 20°C for 15 min and were resuspended in 4 ml methanol and again centrifuged. The combined supernatants were evaporated to dryness under vacuum in a rotatory evaporator. ACC levels were determined by the method of Wachter and others (1999) using the protocol of Lizada and Yang (1979). Residues were resuspended in 2 ml distilled water and then the upper aqueous phase (0.5 ml) obtained by extracting with dichloromethane was mixed with 0.1 ml $HgCl_2$ (80 mmol) in test tubes and sealed with rubber septa. Then 0.2 ml NaOCl solution (40 ml NaOH, 80 ml 12.5% NaOCl solution, 30 ml distilled water) was injected into the tubes, shaken, and incubated for 8 min. One milliliter of the gaseous portion was removed and assayed for ethylene by gas chromatography (GC).

Assay of Enzyme Activity of the Ethylene Biosynthetic Pathway

The protein extracts for measuring in vitro ACS and ACO activity were prepared according to Madhaiyan and others (2007) and Petruzzelli and others (2000). Enzyme extracts for ACS activity were obtained by homogenizing a 1-g sample of pulverized tissues in 4 ml of 100 mmol Na-phosphate (pH 9.0) containing 5 µM pyridoxal phosphate (PLP), 4 mmol 2-mercaptoethanol (2-ME), 1 mmol EDTA, and 10% glycerol in the presence of 1 g polyvinylpolypyrrolidone (PVPP). Ammonium sulfate (35 and 75% saturation) was added to the enzyme extract to obtain precipitation and was resuspended in 2.5 ml of a solution containing 100 mmol Na-phosphate (pH 7.8), 5 µM PLP, 0.5 mmol 2-ME, and 10% glycerol. The preparations of the enzymes were carried out at 4°C (Satoh and others 1997). ACS activity was assayed by incubating a 100-µl enzyme solution (1.29 mg protein) with 100 mmol 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES)–KOH (pH 8.5), 5 µmol PLP, 100 µmol *S*-adenosyl-L-methionine (SAM), and test chemicals at given concentrations in a total volume of 400 µl. After incubation for 15 min at 30°C, the amount of ACC produced was determined as described above.

For assaying the ACO activity, frozen tissues were pulverized in liquid nitrogen and homogenized in 2 ml g^{-1} of extraction buffer consisting of 100 mmol Tris–HCl (pH 7.2), 10% (w/v) glycerol, and 30 mmol sodium ascorbate. The homogenate was centrifuged at 15,000 rpm for 15 min at 4°C. The supernatant obtained was used for the in vitro ACO assay (Malerba and others 1995). Enzyme activity was assayed at 30°C for 15 min in 10-ml screw-cap tubes fitted with a Teflon-coated septum containing 1.5 ml of supernatant, 50 µmol $FeSO_4$, 1 mmol ACC, and 5% (v/v) CO_2. After the incubation period, the quantity of ethylene released into the headspace was determined by GC.

Effect of Salt Stress and ACC Deaminase-producing Halotolerant Bacteria Treatments on Ethylene Biosynthesis of Red Pepper Seedlings

Seeds of red pepper were surface sterilized as described above. Halotolerant bacteria were grown in TSB media and cells were collected and resuspended in 5% NaCl containing N-free media supplemented with 5 mmol of ACC as a nitrogen source and then incubated for 24 h at 30°C with shaking (150 rpm) to induce ACC deaminase activity. Cells were harvested, washed, and resuspended in sterile 0.03 mol $MgSO_4$. Seeds were treated in either halotolerant bacterial suspension (1×10^8 cfu ml^{-1}) or deionized water for 4 h. Seeds not treated with halotolerant bacteria were used as negative controls and seedlings treated only with salt were positive controls. Three replications were used for each treatment. Ethylene production, ACC concentration, and ACS and ACO activity were measured as described above.

Effect of Salt Stress and ACC Deaminase-producing Halotolerant Bacteria Treatments on Growth of Red Pepper Seedlings

Seeds of red pepper were surface sterilized, treated with halotolerant bacteria, and germinated as described above. Eight days after germination each seedling was transplanted into a pot (7.5 × 7.5 cm^2) containing 200 g mixed soil and then fertilized with 10 ml of N-free Hoagland's nutrient solution. The treatments and incubation conditions of red pepper seedlings were the same as described above and four replications per treatment were used. To study the effect of salt stress on the growth of red pepper seedlings, 150 mmol NaCl were applied for 2 weeks, that is, 21-day-old red pepper seedlings, after transfer into individual pots. Thirty-five-day-old red pepper seedlings (that is, 2 weeks after salt stress) were harvested and data on the length and dry weight of the root and shoots were recorded. Based on the growth parameter data obtained, comparisons of the effect of salt stress and halotolerant bacteria inoculation on red pepper growth were performed. Salt tolerance indices (STI) of inoculated and uninoculated plants were also determined, according to Shetty and others (1995), as STI = DWS/DWC or DWH/DWC, where DWS is the dry weight of the plant grown under salt stress, DWH is the dry weight of the plant grown under salt stress with inoculation of halotolerant bacteria, and DWC is the dry weight of the plant grown under control conditions (without salt stress or inoculation of halotolerant bacteria).

Results

Ethylene Production of Red Pepper Seedlings

Ethylene synthesis increased in red pepper seedlings with increasing levels of salt stress (Fig. 1). Ethylene synthesis increased by 77% in stressed red pepper seedlings (150 mmol NaCl) compared to negative controls (Fig. 2). ACC deaminase-producing halotolerant bacteria *Br. epidermidis* RS15, *B. aryabhattai* RS341, and *M. yunnanensis* RS222 treatments reduced ethylene synthesis by 47, 54, and 64%, respectively, compared to positive controls (Fig. 2). *Br. epidermidis* RS15 was found most efficient in reducing stress ethylene, followed by *B. aryabhattai* RS341 and *M. yunnanensis* RS222 (Fig. 2).

Concentration of ACC in Tissue Extracts of Red Pepper Seedlings

The concentration of free ACC in the tissue extracts of red pepper seedlings increased with increasing levels of salt stress (Fig. 1). ACC concentration in red pepper seedlings was 17.6 nmol g^{-1} FW when grown under control conditions but was 56.9 nmol g^{-1} FW when exposed to 150 mmol NaCl stress (Fig. 2). Inoculation of red pepper seedlings by ACC deaminase-producing halotolerant bacteria *Br. epidermidis* RS15, *B. aryabhattai* RS341, and *M. yunnanensis* RS222 reduced the ACC concentration by 47, 48, and 55%, respectively, compared to positive controls (Fig. 2). In general, salt treatments induced a significant increase of ACC concentration in red pepper seedlings (Fig. 2).

ACC Synthase Activity of the Tissue Extracts of Red Pepper Seedlings

The activity of ACC synthase, the enzyme that catalyzes the conversion of SAM to ACC, was measured by GC by measuring the amount of ACC. In red pepper seedlings, ACS activity increased with increasing levels of salt stress (Fig. 1). The in vitro ACS activity was 94.9 nmol ACC g^{-1} FW h^{-1} in red pepper seedlings grown under control conditions, whereas under 150 mmol NaCl stress it was 221.9 nmol ACC g^{-1} FW h^{-1} (Fig. 2). ACS activity in tissues of stressed red pepper seedlings inoculated with *Br. epidermidis* RS15 and *M. yunnanensis* RS222 was 44 and 23% higher, respectively, even compared to positive controls (Fig. 2). Interestingly, seedlings treated with *B. aryabhattai* RS341 showed relatively similar ACS activity compared to that of seedlings exposed to 150 mmol NaCl stress (Fig. 2). In general, salt stress induced a significant increase in ACS activity in red pepper seedlings compared to negative controls, and the activity remained significantly higher even after seedlings were treated with halotolerant bacteria (Fig. 2).

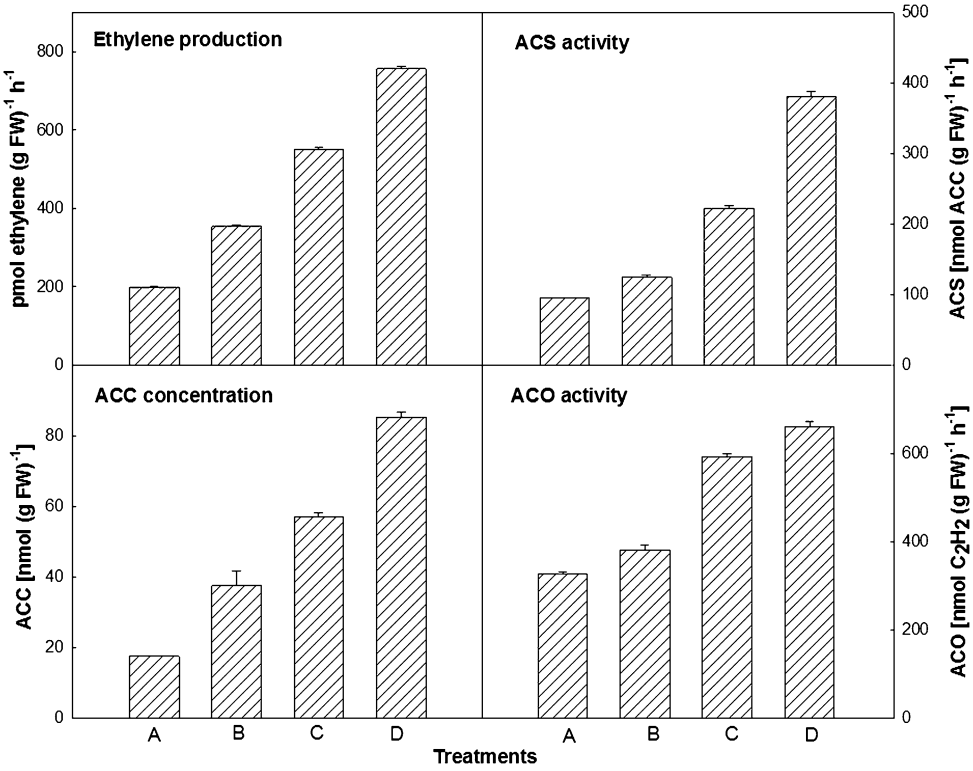

Fig. 1 Effect of different levels of salt stress on ethylene biosynthesis. Treatments: **a** Control (0 NaCl), **b** 100 mmol NaCl, **c** 150 mmol NaCl, and **d** 200 mmol NaCl under greenhouse conditions. Ethylene production was measured after transferring the seedlings to vials and incubating for 4 h. ACC synthase activity was determined from the amount of ACC formed with 100 µmol *S*-adenosyl-L-methionine as substrate. ACC oxidase was determined by supplying 1 mmol ACC as a substrate. The ethylene production and enzyme activities were measured in the tissue extracts of 8-day-old red pepper seedlings grown in soil exposed to 100, 150, and 200 mmol NaCl stress and data recorded after 7 days, that is, 15-day-old red pepper seedlings. Each value represents the mean of three replicates; *error bars* indicate standard deviation

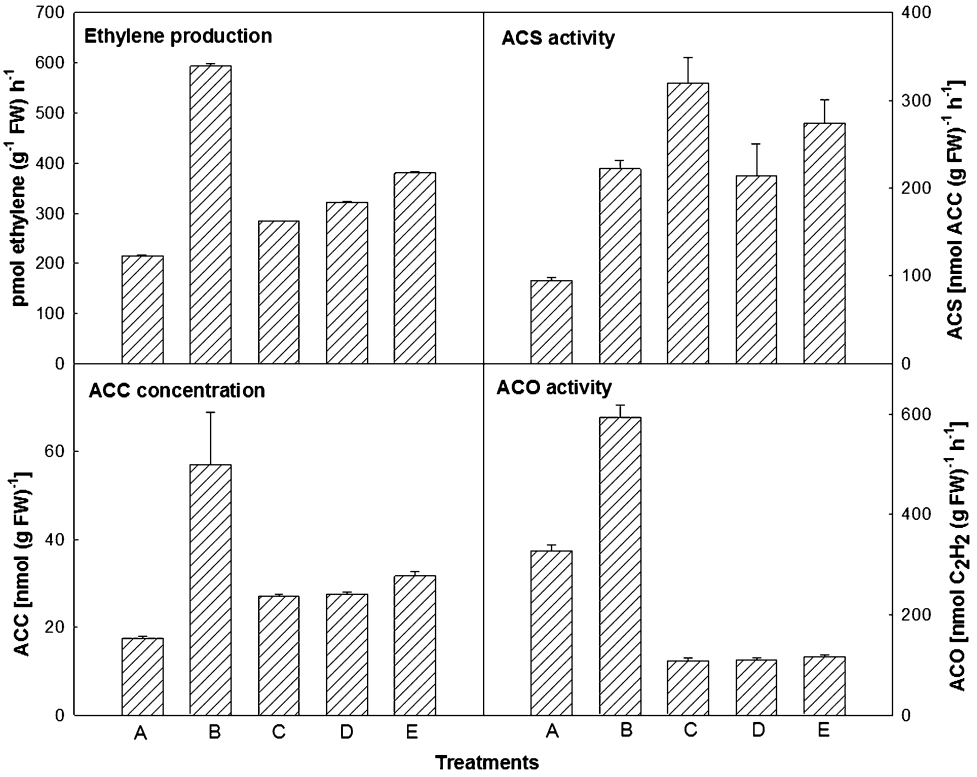

Fig. 2 Effect of salt stress and inoculation with halotolerant bacteria on ethylene biosynthesis. Ethylene production was measured after transferring the seedlings to vials and incubating for 4 h. ACC synthase activity was determined from the amount of ACC formed with 100 µmol *S*-adenosyl-L-methionine as substrate. ACC oxidase was determined by supplying 1 mmol ACC as substrate. The ethylene production and enzyme activities were measured in the tissue extracts of 8-day-old red pepper seedlings grown in soil with the following treatments: **a** 0 mmol NaCl (negative control), **b** 150 mmol NaCl (positive control), **c** *Br. epidermidis* RS15 + 150 mmol NaCl, **d** *B. aryabhattai* RS341 + 150 mmol NaCl, and **e** *M. yunnanesis* RS222 + 150 mmol NaCl. Data recorded after 7 days. Each value represents the mean of three replicates; *error bars* indicate standard deviation

ACC Oxidase Activity of Tissue Extracts of Red Pepper Seedlings

The activity of the ACC oxidase enzyme, which converts ACC into ethylene, could be measured by GC by measuring the amount of ethylene produced. Similar to ethylene production, ACC concentration, and ACC synthase activity, ACC oxidase activity also increased with increasing levels of salt stress (Fig. 1). The in vitro ACO activity of tissue extracts of red pepper seedlings was 327.3 nmol ethylene g^{-1} FW h^{-1} when grown under control conditions but increased to 593.4 nmol ethylene g^{-1} FW h^{-1} in seedlings exposed to 150 mmol NaCl stress, that is, salt stress was 81% higher compared to negative controls (Fig. 2). ACO activity in stressed red pepper seedlings treated with ACC deaminase-producing halotolerant bacteria *Br. epidermidis* RS15, *B. aryabhattai* RS341, and *M. yunnanensis* RS222 was lower by 18, 18, and 19%, respectively, compared to positive controls (Fig. 2). Saline treatment induced ACO activity in red pepper seedlings and treatment with halotolerant bacteria lowered the ACO activity (Fig. 2).

Effect of ACC Deaminase-producing Halotolerant Bacteria Inoculation on Growth of Red Pepper Seedling Under Salt Stress

The growth of 21-day-old red pepper seedlings exposed to 150 mmol NaCl stress for 2 weeks was significantly affected compared to negative controls (Table 1). Salt-stressed plants treated with ACC deaminase-producing halotolerant bacteria grew to a significantly greater extent than those untreated with halotolerant bacteria in respect to length and dry weight of roots and shoots (Table 1). The shoot and root lengths of positive-control (150 mmol NaCl) plants were 46.6 and 51.3% shorter than those of negative-control plants, whereas they were 40.2–48.5% and 37.0–43.3% longer in halotolerant bacteria-treated plants compared to those of positive controls (Table 1). The dry weight of positive-control plants decreased by 53.1% in shoots and 60% in roots compared to negative controls, whereas in halotolerant bacteria-treated plants it increased by 31.4–46.6% in shoots and 44.5–52.6% in roots compared to positive controls. Salinization also decreased the root/shoot ratio of dry matter (12.5%)

Table 1 Effect of salt stress and halotolerant bacteria treatment on growth of red pepper plants under growth chamber conditions

Treatment[a]	Length (cm plant^{-1})[b]		Dry weight (g plant^{-1})[b]		Root/shoot[c]	Dry matter (DM) allocation[c]		Salt tolerance index[c]
	Shoot	Root	Shoot	Root	Dry weight	% in shoot	% in root	
Negative control	23.7 ± 0.6	21.8 ± 0.8	2.6 ± 0.03	1.04 ± 0.03	0.40 ± 0.04	71.2	28.8	
Positive control	12.7 ± 2.4	10.6 ± 0.9	1.2 ± 0.04	0.42 ± 0.03	0.35 ± 0.08	74.4	25.6	0.45 ± 0.04
B. aryabhattai RS341 + 150 mmol NaCl	21.2 ± 0.7	16.9 ± 0.3	1.8 ± 0.02	0.75 ± 0.02	0.42 ± 0.03	70.2	29.8	0.69 ± 0.03
Br. epidermidis RS15 + 150 mmol NaCl	24.6 ± 1.2	17.9 ± 1.5	2.3 ± 0.02	0.88 ± 0.03	0.39 ± 0.03	72.0	27.9	0.87 ± 0.02
M. yunnanensis RS222 + 150 mmol NaCl	22 ± 1.5	18.7 ± 1.2	1.9 ± 0.04	0.78 ± 0.02	0.42 ± 0.04	70.7	29.3	0.73 ± 0.04

21-day-old seedlings were exposed to 150 mmol stress and data recorded after 2 weeks, that is, 35-day-old red pepper seedlings

[a] Treatment: Negative control, seeds and seedlings not treated with salt or halophile; positive control, seeds not treated with halophiles but seedlings exposed to 150 mmol NaCl stress for 2 weeks; inoculation, seeds treated with *Br. epidermidis*, *M. yunnanensis,* and *B. aryabhattai* and seedlings exposed to 150 mmol NaCl stress for 2 weeks

[b] Each value represents the mean ± SD ($n = 3$)

[c] Dry matter allocation, root/shoot ratio of dry matter, and salt tolerance index calculated from mean value of 8 plants (35 days old) for each treatment

compared to negative-control plants (Table 1), whereas *Br. epidermidis* RS15, *M. yunnanensis* RS222, and *B. aryabhattai* RS341 inoculation increased it by 20, 11, and 20% compared to positive controls. Dry matter was around 71.2 and 28.8% in shoots and roots of control plants, whereas in stressed plants it was 74.4 and 25.6%. Similarly, stressed plants inoculated with ACC deaminase-producing halotolerant bacteria exhibited higher tolerance indices compared to positive controls (Table 1).

Discussion

Because an increase in the rate of ethylene production is known to be associated with various environmental stresses, including salt stress (Abeles and others 1992; Mattoo and Suttle 1991; Mayak and others 2004; Morgan and Drew 1997; Wang and others 1990), higher rates of ethylene production as well as concentrations of ACC, the immediate precursor of ethylene, and enzyme activities involved in ethylene metabolism, such as ACS and ACO, in salt-stressed red pepper seedlings were expected. In the present study we found an increase in salt concentration gradually enhanced ethylene production in red pepper seedlings, which is in agreement with earlier studies (Bar and others 1998; Feng and Barker 1992; Lutts and others 1996; Mayak and others 2004). Ethylene sensitivity differs from species to species and cultivar to cultivar (Zapata and others 2007) and even depends on environmental conditions such as light (Mortensen 1989), O_3, CO_2, and so on (Morgan and Drew 1997). When a PGPR contains the enzyme ACC deaminase, the bacterial cells act as a sink for ACC, the immediate biosynthetic precursor of ethylene, thereby lowering plant ethylene levels (Stearns and others 2005). Salt-stressed seedlings treated with ACC deaminase-producing halotolerant bacteria produced lower levels of ethylene compared to positive controls, which is in agreement with earlier studies (Lutts and others 1996; Madhaiyan and others 2006; Mayak and others 2004; Romera and others 1996). Ethylene biosynthesis is regulated by different hormones such as auxins that stimulate production of *de novo* synthesis of ACC synthase followed by an ACC concentration increase (Yi and others 1999). An increase of ACS activity was observed in the *Br. epidermidis* RS15- and *M. yunnanensis* RS222-treated plants. This might have been due to bacterial IAA production which increased the plant IAA. This is in accordance with the model of Glick and others (1998) which suggested that microbial binding to the surface of plant roots results in secretion of IAA which is taken up by the plant. The bacterial IAA together with endogenous plant IAA can induce ACC synthase which converts SAM to ACC. Consequently, ACC exudations are proportional to ACC production (Bayliss and others 1997). In this study ACC formed from methionine, but the action of ACS remained lower in seedlings treated with ACC deaminase-producing halotolerant bacteria. It has been suggested that increased levels of ACC exudations facilitate colonization of ACC deaminase-producing PGPR following utilization, resulting in reduced ACC concentrations (Glick and others 1998). Our results are also supported by the earlier study of Madhaiyan and others (2006) which found that ACC

concentration remained higher in the plants treated with the ACC deaminase mutant *E. cloacae* than in plants treated with the ACC deaminase-producing *Methylobacterium.*

Because ACC is the substrate for ACO, reductions in the substrate level would lead to reduced activities of ACO, subsequently reducing the amount of ethylene, the product of ACC oxidation. Halotolerant bacteria via the enzyme ACC deaminase hydrolyzed ACC into ammonia and α-ketobutyrate. The reduced amount of ACC, the substrate for ACO, consequently resulted in lower amounts of ethylene in stressed plants. Madhaiyan and others (2006) also observed reduced ACO activity in canola seedlings treated with ACC deaminase-producing *Methylobacterium* sp. The increased ethylene production was correlated with the increased concentration of ACC in petals (Bufler and others 1980), increased activities of ACC synthase and ACC oxidase (Woodson and others 1992), and expression of both ACS (Park and others 1992) and ACO genes in senescing petals (Woodson and others 1992).

However, bacterial inoculation has been reported to have a positive influence on various plant-growth parameters, including root and shoot length, seedling vigor, and dry biomass. ACC deaminase-producing bacteria have been reported to prevent the inhibition of root elongation by decreasing the level of growth-limiting ethylene through hydrolytic cleavage (deaminase activity) of the ethylene biosynthesis precursor ACC (Cheng and others 2007; Glick and others 1998; Madhaiyan and others 2006; Mayak and others 2004).

Studies using AVG, an inhibitor of ACS, showed promotive effects as a result of reducing ethylene production (Khalafalla and Hattori 2000; Madhaiyan and others 2006, 2007). However, other studies involving ACC deaminase-negative *Pseudomonas* sp. have shown significantly increased root length, which suggests that the results of other plant-growth-promoting traits such as IAA, SA, cytokinin, and gibberellic acid should also be considered in addition to just ACC deaminase activity (Cattelan and others 1999; Penrose and Glick 2003).

The present study demonstrated that ACC deaminase-producing halotolerant bacteria reduced ethylene biosynthesis by breaking down ACC into ammonia and α-ketobutyrate, resulting in reduced ethylene biosynthesis and inhibiting the negative effects of stress ethylene. ACS activity increased in red pepper seedlings and ACC concentration decreased with inoculation by halotolerant bacteria in red pepper seedlings under salt stress. Thus, to conclude, the promotion of plant growth by halotolerant bacteria in the presence of a growth inhibitory level of salt is a consequence of the reduced stress level ethylene synthesis possibly due to the activity of the ACC deaminase enzyme. However, further study is needed to uncover what are the additional traits or factors involved in plant growth promotion under salt stress by ACC deaminase-producing halotolerant bacteria.

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