Effects of 1-aminocyclopropane-1-carboxylate-deaminase–Producing Bacteria on Perennial Ryegrass Growth and Physiological Responses to Salinity Stress

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ABSTRACT. The accumulation of 1-aminocyclopropane-1-carboxylate (ACC), which is a precursor for ethylene production, in plant roots exposed to salinity stress can be detrimental to plant growth. The objectives of this study were to determine whether inoculating roots with bacteria containing deaminase enzymes that break down ACC (ACC-deaminase) could improve plant tolerance to salinity in perennial ryegrass (*Lolium perenne*) and to examine growth and physiological factors, as well as nutrition status of plants affected by the ACC-deaminase bacteria inoculation under salinity stress. Plants of perennial ryegrass (cv. Pangea) were inoculated with either *Burkholderia phytofirmans* PsJN or *Burkholderia gladioli* RU1 and irrigated with either fresh water (control) or a 250 mM NaCl solution to induce salinity stress. The bacterium-inoculated plants had less ACC content in shoots and roots under both nonstressed and salinity conditions. Salinity stress inhibited root and shoot growth, but the bacterium-inoculated plants exhibited higher visual turf quality (TQ), tiller number, root biomass, shoot biomass, leaf water content, and photochemical efficiency, as well as lower cellular electrolyte leakage (EL) under salinity stress. Plants inoculated with bacteria had lower sodium content and higher potassium to sodium ratios in shoots under salinity stress. Shoot and root nitrogen content and shoot potassium content increased, whereas shoot and root calcium, magnesium, iron, and aluminum content all decreased due to bacterial inoculation under salinity treatment. ACC-deaminase bacteria inoculation of roots was effective in improving salinity tolerance of perennial ryegrass and could be incorporated into turfgrass maintenance programs in salt-affected soils.

Salinity is a major stress limiting plant growth in areas with saline soils or irrigated with poor quality water. Salinity stress can impose cellular and physiological damages including osmotic stress, ion toxicity, and nutrient disturbances (Alshammary et al., 2004). Salinity stress may cause changes in hormone metabolism in plants, including the promotion of ethylene production (Morgan and Drew, 1997). Increases in the production of an ethylene precursor, ACC, have been widely reported to be associated with salinity stress in leaves and roots of various plant species (Arbona et al., 2003; Ghanem et al., 2008; Gómez-Cadenas et al., 1998; Kukreja et al., 2005; Zapata et al., 2004). Excessive ethylene in plants exposed to stresses, including salinity stress, adversely affects shoot and root growth, mainly by induction of leaf or root maturation and senescence (Abeles et al., 2012; Morgan and Drew, 1997). Approaches that can suppress excessive accumulation of stress-induced ethylene may be effective to mitigate stress damages.

Some plant growth–promoting bacteria (PGPB) such as *B. phytofirmans* and *B. gladioli* contain deaminase enzymes that use ACC as a nitrogen source, breaking down ACC and reducing ACC availability for ethylene synthesis (Saleem et al., 2007). The inoculation of plants with ACC-deaminase–producing bacteria was reported to promote shoot and root growth under salinity stress in cereals and horticultural crops,

such as rice [*Oryza sativa* (Bal et al., 2013)], maize (*Zea mays*), wheat [*Triticum aestivum* (Nadeem et al., 2007, 2010)], tomato [*Solanum lycopersicum* (Mayak et al., 2004)], cucumber [*Cucumis sativus* (Gamalero et al., 2010)], and red pepper [*Capsicum annuum* (Siddiquee et al., 2012)]. Several studies reported inoculation of plants with ACC-deaminase bacteria increased leaf relative water content (RWC) (Ahmad et al., 2013; Nadeem et al., 2007, 2010), chlorophyll content (Bal et al., 2013; Mayak et al., 2004; Nadeem et al., 2007), and water use efficiency (Mayak et al., 2004) under salinity stress. The improved growth of transgenic canola (*Brassica napus*) with ACC deaminase activity under salinity stress suggested that ethylene is responsible for the inhibited growth of salinized plants (Sergeeva et al., 2006). Despite of reports on ACC-deaminase bacteria enhancing plant tolerance to salinity stress, the underlying physiological mechanisms are still not well understood.

Salinity stress becomes increasingly significant concern in turfgrass management due to the decline in the availability of fresh water and the increasing use of recycled water for irrigation (Carrow and Duncan, 1998). ACC-deaminase bacteria could be potentially useful to be incorporated into turfgrass management programs to combat salinity stress, assuming there exist positive effects for turfgrass. However, effects of ACC-deaminase bacteria on turfgrass growth and salinity tolerance have not been previously reported. Furthermore, limited information is available on physiological factors involved with ACC-deaminase bacteria effects, which deserve investigation. Therefore, the objectives of this study were to determine whether ACC-deaminase–producing bacteria could promote growth and

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salinity tolerance for a widely used turfgrass species, perennial ryegrass, and to investigate physiological effects of ACC-deaminase-producing bacteria inoculation on perennial ryegrass responses to salinity stress.

Materials and Methods

PLANT MATERIALS AND GROWTH CONDITIONS. Perennial ryegrass (cv. Pangea) plants were collected from the turfgrass research farm of Rutgers University at North Brunswick, NJ, and transplanted into pots on 1 Oct. 2013. Tillers of similar sizes were surface-sterilized by soaking in 1% sodium hypochlorite for 30 s then rinsed twice in sterile water. Ten tillers were transplanted into each pot (6 cm diameter and 50 cm deep) filled with sterile fritted clay (Profile Products, Deerfield, IL). Plants were established for 28 d during Oct. 2013 in a greenhouse with an average temperature of 21 °C day/15 °C night and 710 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetically active radiations (PAR) from natural sunlight and supplemental lighting. Plants were irrigated daily with sterile water and fertilized weekly with sterile half-strength Hoagland's solution (Hoagland and Arnon, 1950). Following establishment, plants were transferred to growth chambers (Environmental Growth Chamber, Chagrin Falls, OH) and allowed to acclimate for 4 d before bacterial inoculation and subsequent salinity treatment. The controlled-environment growth chambers were set to maintain 23 °C day/18 °C night, 680 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR, 60% relative humidity, and 12-h photoperiod.

BACTERIAL PREPARATION AND INOCULATION. Two ACC-deaminase-producing bacteria species, *B. phytofirmans* strain PsJN and *B. gladioli* RU1 were used to inoculate perennial ryegrass plants. PsJN has been previously reported having ACC-deaminase activities (Mitter et al., 2013). RU1 is an ACC-deaminase-producing strain isolated from the soil established with turfgrass at the research farm of Rutgers University. The isolation was based on a minimum medium method described by Penrose and Glick (2003). Bacterial cultures were revived from frozen stock vials stored at -80 °C by streaking on nutrient agar plates. Single colonies were picked and inoculated in lysogeny broth and incubated at 23 °C on a water-bath shaker for 48 h. Bacterial suspensions were centrifuged at 8000 g_n for 10 min at 4 °C then resuspended in deionized water. The centrifuge and resuspension process was repeated twice to remove the lysogeny broth. The prepared bacterial suspension was adjusted to optical density of 1.0. Plants were inoculated by soil drenching with 30 mL prepared bacterial inoculum into each pot twice at an interval of 6 h. The control group for the bacterial inoculation treatment was watered with 30 mL of deionized water. At 7 and 14 d of inoculation, bacteria were isolated from roots of plants inoculated through soil drenching and detected ACC-deaminase activity of bacteria, which indicated successful inoculation of both strains.

SALINITY TREATMENT AND EXPERIMENTAL DESIGN. Salinity treatment was initiated 1 d following bacterial inoculation. Plants in each pot received 50 mL sterile NaCl solution daily for the duration of the experiment. NaCl treatment was increased at 2-d intervals from 20, 40, 80, 160 to 250 mM to avoid initial salinity shock. Plants were subjected to 250 mM salinity irrigation for 21 d. The experimental design was completely randomized design with two factors (salinity treatment and bacterial inoculation). Each treatment consisted of four replicates and three subsamples (containers) with a total of 12 containers (multiple plants in each container). Four replicates

Table 1. Effects of bacterial inoculation on turf quality of perennial ryegrass at 0, 10, and 20 d of nonstressed control or salinity treatment.

Treatment ^z	Turf quality (1–9 scale) ^y		
	0 d	10 d	20 d
Control	9.0 a ^x	9.0 a	7.8 b
Control + PsJN	9.0 a	8.8 ab	8.0 ab
Control + RU1	9.0 a	8.9 ab	8.3 a
Salinity	9.0 a	8.5 c	6.2 d
Salinity + PsJN	9.0 a	8.8 abc	6.8 c
Salinity + RU1	9.0 a	8.5 bc	6.8 c

^zPsJN treatments were inoculated with *Burkholderia phytofirmans* PsJN. RU1 treatments were inoculated with *Burkholderia gladioli* RU1.

^y1 = brown and desiccated turf; 6 = minimal acceptable level; 9 = green and dense turf.

^xValues are means of four replicates. Values with the same letter within each column (noninoculated, PsJN, and RU1) indicated no significant difference based on Fisher's protected least significance different test at $P \leq 0.05$.

Fig. 1. Tiller number of *Burkholderia gladioli* RU1- and *Burkholderia phytofirmans* PsJN-inoculated and noninoculated perennial ryegrass under (A) nonstressed control condition and (B) salinity condition. Vertical bars indicate Fisher's protected least significant difference values ($P \leq 0.05$) for comparison between treatments at a given day of treatment where significant differences were detected.

for each treatment were placed in four different growth chambers, and containers of plants were randomly placed inside each growth chamber. In addition, all containers were relocated among four growth chambers every 3 d to avoid possible confounding effects of chamber environmental variations.

Fig. 2. (A) Shoot biomass and (B) root biomass of *Burkholderia gladioli* RU1- and *Burkholderia phytofirmans* PsJN-inoculated and noninoculated perennial ryegrass at 10 and 20 d of nonstressed control and salinity conditions. Columns marked with different letters indicate significant differences between treatments within each inoculation group (noninoculated, PsJN inoculated, and RU1 inoculated) based on Fisher's protected least significant difference test at $P \leq 0.05$.

PHYSIOLOGICAL ANALYSES. Leaf EL was measured as an indicator of cellular membrane stability according to the procedure by Blum and Ebercon (1981). About 0.2 g fresh leaves were collected, rinsed with deionized water to remove exogenous solutes, and placed in a test tube containing 30 mL deionized water. Tubes were placed on a conical flask shaker for 12 h and the initial conductance (C_i) measured using a conductivity meter (model 132; YSI, Yellow Springs, OH). Leaf samples were killed by autoclaving at 120 °C for 20 min and shaking for 12 h. The maximal conductance of killed tissue (C_{max}) was then measured. EL was calculated using the formula (%) = $(C_i/C_{max}) \times 100$.

RWC was measured according to the procedure by Barrs and Weatherley (1962). Leaf RWC was calculated based on leaf fresh weight (FW), turgid weight (TW), and dry weight (DW) using the formula (%) = $100 \times [(FW - DW)/(TW - DW)]$. FW of leaves was determined with a mass balance immediately after detaching leaves from the plant. Samples were then wrapped in tissue paper and submerged in deionized water for 12 h at 4 °C. Leaf samples were removed from the water, blotted dry, and again weighed for TW. Following a drying period of 3 d at 80 °C, samples were

weighed a final time for DW. Leaf photochemical efficiency was estimated by measuring chlorophyll fluorescence expressed as the ratio of variable to maximum fluorescence (F_v/F_m) with a fluorescence induction monitor (OS 1FL; Opti-Sciences, Hudson, NH). Leaves were dark adapted for 30 min before F_v/F_m was measured.

ACC DETERMINATION. ACC content was determined according to the method of Lizada and Yang (1979). About 0.1 g of fresh leaf tissue was ground into powder with liquid nitrogen and dissolved in 1.5 mL ethanol. The sample was then centrifuged at 10,000 g_n for 15 min at 4 °C and the supernatant was evaporated in a vacuum at 50 °C. The sample was added with 0.75 mL deionized H_2O and 0.75 mL chloroform and then vortexed and centrifuged at 10,000 g_n for 15 min at 4 °C. A 0.5 mL of the water phase extract was transferred to glass tube with rubber cap affixed, 10 $\mu L \times 0.1$ M $HgCl_2$ was added, and the volume was brought up to 0.8 mL with water. A 0.2 mL ice cold mixture (v/v = 2:1) of commercial bleach (8% NaOCl) and saturated NaOH was injected by a syringe and the glass tube was vortexed. Following 3-min incubation on ice, a 1-mL air sample was withdrawn using a syringe and injected into a gas chromatograph (GC-8A; Shimadzu Scientific Instruments, Columbia, MD) (Watkins and Frenkel, 1987).

SHOOT AND ROOT GROWTH ANALYSES. Visual evaluation of TQ was performed biweekly during the salinity treatment. TQ was rated on a scale of 1 to 9, with 1 being brown and desiccated turf, 6 being the minimal acceptable level, and 9 being green and dense turf. Ratings were based on parameters such as uniformity, visual attractiveness, leaf color, and canopy density (Beard, 1972). Tiller density was determined by manually counting the numbers of tillers in each pot every 5 d. Shoot and root DWs were measured at 10 and 20 d of salinity treatment. Roots were washed free of fritted clay and severed from shoots by destructively sampling 10 different plants at 10 or 20 d of salinity treatment. All tissues were dried at 80 °C for 3 d and weight measured using a mass balance. Root morphological parameters were analyzed on harvest at 20 d of salinity treatment. Roots were washed free of fritted clay, stained with 1% crystal violet solution, and scanned with a digital scanner (Expression 1680; Epson, Long Beach, CA) to generate high-definition digital images. Images were analyzed using WinRHIZO Basic V.2002 software (Regent Instruments, QC, Canada) for root length, volume, surface area, and diameter.

SHOOT AND ROOT NUTRIENT ANALYSIS. Roots were washed free of fritted clay and severed from shoots at 20 d after salinity initiation. They were washed with deionized water and dried at 80 °C for 3 d. The dry plant samples were ground with liquid nitrogen and passed through a 2-mm-mesh sieve. About 0.2-g samples were analyzed for nutrient content in shoots and roots. Nitrogen content was determined using the combustion method of Horneck and Miller (1998). The content of P, K, Ca, Mg, Mn, Fe, Cu, B, Al, Zn, and Na was measured by the dry ash method (Miller, 1998).

STATISTICAL ANALYSIS. Main effects of salinity or bacterial inoculation and their interactions were determined by analysis of variance according to the general linear model procedure of a statistical program (SAS version 9.0; SAS Institute, Cary, NC). Differences between treatment means were separated by Fisher's protected least significance difference test at the 0.05 P level.

Results

SHOOT AND ROOT GROWTH AS AFFECTED BY THE INOCULATION WITH ACC-DEAMINASE-PRODUCING BACTERIA.

No significant differences in TQ were observed between the bacterium-inoculated plants and the noninoculated plants at either 10 or 20 d of nonstressed conditions (Table 1). Salinity caused decrease of TQ in noninoculated plants at 10 and 20 d of salinity treatment, and in inoculated plants only at 20 d. Under salinity treatment, an increase in TQ (9.7%) was detected in the inoculated plants with both bacteria species at 20 d, compared with TQ of the noninoculated plants. No significant difference in TQ was observed between the two bacterial treatments under salinity stress. Bacterial inoculation increased the number of tillers under both non-stressed and salinity conditions, but to a greater extent under non-stressed conditions, particularly with PsJN inoculation, which was as much as 2-fold higher than that of the noninoculated controls (Fig. 1A and B). Both bacterial inoculations showed significant positive effects on shoot and root biomass accumulation at 10 and 20 d of salinity stress (more than 2-fold higher), compared with the noninoculated plants. Shoot biomass of PsJN-inoculated plants was significantly higher than that of RU1-inoculated plants at 10 d of salinity conditions (18%) and at 20 d of both nonstressed (14%) and salinity conditions (18%) (Fig. 2A). For root biomass, the difference between PsJN and RU1 inoculation was not significant at 10 d of both non-stressed and salinity conditions and at 20 d of salinity conditions. At 20 d, root biomass of PsJN-inoculated plants was significantly lower (21%) than that of RU1-inoculated plants under nonstressed conditions (Fig. 2B).

Both bacterial treated plants had significantly higher root length (25% for both inoculants) and root volume (34% and 45% for RU1 and PsJN, respectively) under nonstressed conditions. Under salinity condition, the difference was significant only between RU1-inoculated and the noninoculated control, but not between PsJN and its noninoculated control (Fig. 3A and C). Both bacterial species increased root surface area under nonstressed conditions (36% and 48% for RU1 and PsJN, respectively), and only RU1 inoculation significantly increased root surface area (80%) under salinity conditions (Fig. 3B). No difference in root diameter was observed among bacterial treatments and the noninoculated control under both nonstressed and salinity conditions (Fig. 3D).

Both bacterial species increased root surface area under nonstressed conditions (36% and 48% for RU1 and PsJN, respectively), and only RU1 inoculation significantly increased root surface area (80%) under salinity conditions (Fig. 3B). No difference in root diameter was observed among bacterial treatments and the noninoculated control under both nonstressed and salinity conditions (Fig. 3D).

PHYSIOLOGICAL EFFECTS OF THE INOCULATION WITH ACC-DEAMINASE-PRODUCING BACTERIA. Leaf photochemical efficiency declined under salinity conditions (Fig. 4B). Both bacterial inoculated plants had higher F_v/F_m under both non-stressed and salinity conditions compared with the noninoculated

Fig. 3. (A) Root total length, (B) root surface area, (C) root volume, and (D) root average diameter of *Burkholderia gladioli* RU1- and *Burkholderia phytofirmans* PsJN-inoculated and noninoculated perennial ryegrass under nonstressed control and salinity conditions. Columns marked with different letters indicate significant differences between treatments within each inoculation group (noninoculated, PsJN-inoculated, and RU1-inoculated) based on Fisher's protected least significant difference test at $P \leq 0.05$.

control plants. No significant differences existed between the two bacterial inoculations under either nonstressed or salinity conditions (Fig. 4A and B).

Leaf EL was lower in bacterial inoculated plants than the noninoculated plants under either nonstressed or salinity conditions (Fig. 5A and B). There was no difference in leaf EL between plants inoculated with the two bacteria species under nonstressed conditions (Fig. 5A). After 15 d of salinity treatment, EL of PsJN-inoculated plants was significantly higher than RU1-inoculated plants (Fig. 5B).

Under nonstressed conditions, RWC remained around 90% and no significant differences existed among bacteria-inoculated plants and noninoculated control plants (Fig. 6A). Under salinity conditions, RWC of the noninoculated plants were significantly lower than that of plants inoculated with either bacteria strain (Fig. 6B). There was no significant difference in RWC between the two bacteria species inoculations during most of the treatment period under salinity conditions.

Both shoot and root ACC contents under salinity treatment were higher (up to 2-fold) than those of the nonstressed plants (Fig. 7A and B). Shoot ACC content was significantly lower (20%) in plants inoculated with PsJN at 20 d of nonstressed conditions (Fig. 7A). Under salinity conditions, shoot ACC contents were significantly lower (40% and 36% for RU1 treatments at 10 and 20 d, respectively, 43% and 38% for PsJN treatments at 10 and 20 d, respectively) in the two bacteria-treated plants than in the noninoculated plants (Fig. 7A). No significant difference in shoot ACC content was detected between plants inoculated with the two bacteria species under salinity conditions (Fig. 7A). In root tissues (Fig. 7B), the ACC content was not significantly affected by bacteria inoculation

Fig. 4. Leaf photochemical efficiency (F_v/F_m) of *Burkholderia gladioli* RU1- and *Burkholderia phytofirmans* PsJN-inoculated and noninoculated perennial ryegrass under (A) nonstressed control condition and (B) salinity condition. Vertical bars indicate Fisher's protected least significant difference values ($P \leq 0.05$) for comparison between treatments at a given day of treatment where significant differences were detected.

under nonstressed conditions. Under salinity conditions, root ACC content of PsJN- or RU1-inoculated plants was significantly lower (23% and 38% for RU1 treatments at 10 and 20 d, respectively, 24% and 32% for PsJN treatments at 10 and 20 d, respectively) than that in the noninoculated plants. Plants inoculated with the two bacteria species had no significance differences in root ACC content under either nonstressed or salinity conditions (Fig. 7B).

SHOOT AND ROOT NUTRIENT STATUS AS AFFECTED BY THE INOCULATION WITH ACC-DEAMINASE-PRODUCING BACTERIA. The content of Na in shoot tissues increased dramatically under salinity treatment in both shoots (from 0.08% to 3.98%) and roots (from 0.14% to 0.38%) of the control plants (Table 2). In bacterial inoculated plants, a significant lower Na content was observed in shoot tissues under both nonstressed (50% and 37% lower for RU1 and PsJN, respectively) and salinity conditions (77% and 55% lower for RU1 and PsJN, respectively), compared with that in the noninoculated plants. No significant difference in root Na content was detected between bacteria-inoculated and noninoculated plants under salinity conditions. For K content, shoots of both inoculated plants had higher levels than the noninoculated plants under both nonstressed (127% and 140% for PsJN and RU1, respectively) and salinity conditions (147% and 154% for PsJN and RU1, respectively)

Fig. 5. Electrolyte leakage of *Burkholderia gladioli* RU1- and *Burkholderia phytofirmans* PsJN-inoculated and noninoculated perennial ryegrass under (A) nonstressed control condition and (B) salinity condition. Vertical bars indicate Fisher's protected least significant difference values ($P \leq 0.05$) for comparison between treatments at a given day of treatment where significant differences were detected.

(Table 2). Roots of PsJN-treated plants had higher K content (44%) than the noninoculated plants under salinity conditions (Table 2). Bacteria-inoculated plants had greater K/Na ratio in shoots under both nonstressed (2.7- and 2.2-fold for RU1 and PsJN, respectively) and salinity conditions (6.7- and 3.3-fold for RU1 and PsJN, respectively).

The inoculation of plants with two bacterial species had differential effects on macronutrients and micronutrients under nonstressed and salinity conditions. For N content, there was a significant increase in both shoots (14% and 21% for RU1 under nonstressed and salinity condition, respectively, 15% and 20% for PsJN under nonstressed and salinity condition, respectively) and roots (12% and 17% for RU1 under nonstressed and salinity condition, respectively, 22% and 23% for PsJN under nonstressed and salinity condition, respectively) in the inoculated plants compared with the noninoculated plants under either nonstressed or salinity conditions (Table 3). No effects of bacterial inoculation were observed on shoot and root P content under either nonstressed or salinity conditions (Table 3). For Ca and Mg content, the effect of bacterial inoculation only showed significance under salinity conditions with significantly lower content in bacterial inoculated plants compared

Fig. 6. Relative water content of *Burkholderia gladioli* RU1- and *Burkholderia phytofirmans* PsJN-inoculated and noninoculated perennial ryegrass under (A) nonstressed control condition and (B) salinity condition. Vertical bars indicate Fisher's protected least significant difference values ($P \leq 0.05$) for comparison between treatments at a given day of treatment where significant differences were detected.

with the noninoculated plants in both shoots (31% and 25% lower for RU1 in Ca and Mg content, respectively, 22% and 14% lower for PsJN in Ca and Mg content, respectively) and roots (23% and 25% lower for both RU1 and PsJN in Ca and Mg content, respectively).

Shoot Fe content was significantly lower in bacterial inoculated plants than noninoculated control under both nonstressed (32% and 33% lower for RU1 and PsJN, respectively) and salinity conditions (35% and 73% lower for RU1 and PsJN, respectively) (Table 3). PsJN-inoculated plants had significantly lower root Fe content than the noninoculated plants under both nonstressed (35% lower) and salinity conditions (39% lower), while the difference in root Fe content between RU1 inoculated plants and the noninoculated control was not significant. Al content of both shoots and roots were significantly lower in both RU1- (68% and 27% for shoots and roots, respectively) and PsJN- (30% and 36% for shoots and roots, respectively) treated plants than those in the noninoculated control under salinity condition. Under nonstressed conditions, shoot Al content of RU1-inoculated plants was significant lower (25%) than that of the noninoculated control; root Al content of PsJN-inoculated plants was significant lower (23%) than that of the noninoculated control

Fig. 7. (A) Shoot 1-aminocyclopropane-1-carboxylic acid (ACC) content and (B) root ACC content of *Burkholderia gladioli* RU1- and *Burkholderia phytofirmans* PsJN-inoculated and noninoculated perennial ryegrass under nonstressed control and salinity conditions. Columns marked with different letters indicate significant differences between treatments within each inoculation group (noninoculated, PsJN inoculated, and RU1 inoculated) based on Fisher's protected least significant difference test at $P \leq 0.05$.

(Table 3). Bacterial inoculation had no significant effects on Mn and Zn content in roots. Shoot Mn content was significantly lower (29%) in PsJN-inoculated plants compared with the noninoculated plants under nonstressed condition. Shoot Zn content was significantly lower in both bacterial inoculations (34% and 33% lower for RU1 and PsJN, respectively) under nonstressed condition and in RU1-inoculated plants (26%) under salinity condition compared with the noninoculated plants (Table 3).

Discussion

As discussed in the introduction, increased ethylene production under stress conditions can be detrimental to plant growth. In this study, perennial ryegrass produced higher content of ACC under salinity stress than those under nonstressed conditions; the bacterial inoculated perennial ryegrass showed significantly lower ACC content in both root tissues and shoot tissues, suggesting that ACC-deaminase-producing bacteria suppressed ACC accumulation in plant tissues. Siddikee et al. (2012) found red pepper seedlings inoculated by ACC-deaminase-producing bacteria showed significant reduction in levels of ACC under salinity condition. Other related

Table 2. Content of K and Na and K/Na ratio in shoots and roots of perennial ryegrass plants under nonstressed control or salinity treatment at 20 d after treatment.

	Treatment		K (10 ⁴ mg·kg ⁻¹)	Na (10 ⁴ mg·kg ⁻¹)	K/Na (ratio)
Shoot	Control	Noninoculated	1.36 b ^z	0.08 a	16.87 b
		PsJN ^y	1.73 a	0.05 b	37.39 a
		RU1 ^x	1.91 a	0.04 b	45.33 a
	Salinity	Noninoculated	1.07 b	3.98 a	0.27 b
		PsJN	1.57 a	1.81 b	0.88 a
		RU1	1.65 a	0.93 c	1.81 a
Root	Control	Noninoculated	1.76 a	0.14 b	12.68 a
		PsJN	1.88 a	0.17 a	11.63 a
		RU1	1.30 b	0.15 ab	8.78 b
	Salinity	Noninoculated	1.38 b	0.38 a	3.68 ab
		PsJN	2.00 a	0.48 a	4.22 a
		RU1	1.34 b	0.39 a	3.40 b

^zValues are means of four replicates. Values with the same letter within each column (noninoculated, PsJN, and RU1) indicated no significant difference based on Fisher's protected least significant difference test at $P \leq 0.05$.

^yPsJN treatments were inoculated with *Burkholderia phytofirmans* PsJN.

^xRU1 treatments were inoculated with *Burkholderia gladioli* RU1.

studies that measured ethylene production rate instead of ACC content also found that ACC-deaminase-producing bacteria decreased ethylene production and improved stress tolerance (Grichko and Glick, 2001; Mayak et al., 2004; Siddikee et al., 2011). These results suggested that ACC-deaminase-producing bacteria could effectively reduce stress-induced ACC accumulation, which likely contribute to their positive growth and physiological effects on improving salinity tolerance in perennial ryegrass.

In our study, plants inoculated with *B. phytofirmans* PsJN or *B. gladioli* RU1 also showed an increase in TQ, tiller formation, and shoot biomass, indicating both ACC-deaminase bacterial strains could improve shoot growth of perennial ryegrass through the bacterial hydrolysis of ACC. In addition, a more extensive root system was observed for plants inoculated with either bacterial species under nonstressed conditions, with higher total root length and root volume although root diameter was unaffected. Root growth promotion effects were also observed under salinity conditions in plants inoculated with RU1. This is consistent with the previous reports of increased root length and surface area in other plant species by inoculating ACC-deaminase-producing bacteria under salinity conditions (Bal et al., 2013; Gamalero et al., 2010; Siddikee et al., 2011, 2012). This better developed root system could enhance water and nutrient uptake under stress conditions.

Physiological analyses, measured as RWC, EL, and F_v/F_m , indicated that the inoculation of perennial ryegrass with PsJN or RU1 may also help to maintain better cellular hydration and membrane stability, and greater photochemical efficiency under salinity stress. The increased RWC by PGPB under salinity stress has also been reported in maize (Nadeem et al., 2007), wheat (Nadeem et al., 2010), and mung bean [*Vigna radiata* (Ahmad et al., 2013)]. Mayak et al. (2004) reported increased water use efficiency of PGPB-inoculated plants. Naveed et al. (2014) also reported an increase of F_v/F_m in PGPB-inoculated maize under normal growth conditions. Lowering EL has also been reported in PGPB-inoculated peanut (*Arachis hypogaea*) under salinity by Shukla et al. (2012). Our results suggested that the reduction in ACC content and thus the subsequent decrease in ethylene accumulation under salinity stress through

ACC-deaminase bacteria may have promoted physiological tolerance of perennial ryegrass to salinity stress.

Maintaining ion homeostasis, such as increasing K^+/Na^+ ratio, can detoxify the adverse effects of Na^+ accumulation in plants exposed to salinity stress (Hamdia et al., 2004). Na^+ uptake competes with K^+ acquisition due to their physicochemical similarities in plants (Maathuis and Amtmann, 1999). The accumulation of K^+ is critical for plant tolerance to salinity stress by balancing the osmotic stress due to the accumulation of Na^+ in cytosol or vacuoles to maintain cell turgor and exclude Na^+ from entering into cells exposed to salt stress (Maathuis and Amtmann, 1999). Mayak et al. (2004) reported that the main effect of the ACC-deaminase-producing PGPB inocula-

tion was an increase in the uptake of K, which plays an important role in balancing ψ_s of the vacuole (Hu et al., 2011). Nadeem et al. (2007, 2010) also reported a higher K^+/Na^+ ratio in ACC-deaminase-producing PGPB-inoculated maize and wheat under salinity stress. In our study, inoculated perennial ryegrass with either bacterial strain had a significant higher K^+/Na^+ ratio in shoot tissues under both nonstressed and salinity conditions. In addition, salinity caused increases in shoot and root Na content but shoot Na content was lower in bacterial inoculated plants compared with that in noninoculated plants; root Na content did not differ between the inoculated and the noninoculated plants. These results indicated that the ACC-deaminase bacteria may affect shoot exclusion or extrusion of Na^+ and help to maintain K^+ and Na^+ balance to minimize the toxic effects of Na^+ . However, the mechanisms of how lowered ACC production in plant tissues by ACC-deaminase bacteria affect Na accumulation and K balance are not clear, which deserves further investigation.

The content of other macronutrients, including N, Ca, and Mg, were also altered by the bacterial inoculation in addition to changes in Na and K content. The ACC-deaminase bacterial inoculation also increased N content in shoots and roots. The increased K and N content could be due to increased root growth for nutrient uptake, which was also reflected in the increased TQ. In contrast to K and N, the content of Ca and Mg decreased with bacterial inoculation, although the lower level of Ca or Mg was not decreased enough causing deficient symptoms. The underlying factors for the suppression Ca and Mg accumulation by the inoculation of ACC-deaminase bacteria are unknown, despite their positive effects on improving salinity tolerance.

For micronutrients, excessive accumulation of Fe, Al, Mn, and Zn can be detrimental to plant growth: excess Fe could inhibit the uptake of other nutrients such as P and K; excess Al could interfere root cell division, decrease P availability and root respiration; excess Mn could result in root growth inhibition; excess Zn could show negative effects on mineral nutrition and enzyme activities (Foy et al., 1978). The sufficient ranges of different nutrient elements vary with plant species and soil and their environmental conditions, but it is typical within

Table 3. Mineral nutrient content in shoots and roots of perennial ryegrass plants under nonstressed control or salinity treatment at 20 d after treatment.

Shoot	Treatment ^z		N (10 ⁴ mg·kg ⁻¹)	P (10 ⁴ mg·kg ⁻¹)	Ca (10 ⁴ mg·kg ⁻¹)	Mg (10 ⁴ mg·kg ⁻¹)	Mn (mg·kg ⁻¹)	Fe (mg·kg ⁻¹)	Al (mg·kg ⁻¹)	Zn (mg·kg ⁻¹)
	Control	Noninoculated	1.39 b ^y	0.24 a	0.49 a	0.23 a	967.09 a	1,088.07 a	873.44 a	31.15 a
Root	Salinity	PsJN	1.60 a	0.25 a	0.48 a	0.23 a	685.76 b	726.93 b	703.19 ab	20.85 b
		RUI	1.58 a	0.29 a	0.45 a	0.23 a	809.68 ab	743.68 b	654.86 b	20.67 b
		Noninoculated	1.35 b	0.28 a	0.71 a	0.28 a	801.54 a	1,428.26 a	1,162.96 a	30.06 a
		PsJN	1.62 a	0.26 a	0.55 b	0.24 b	638.53 a	935.33 b	808.75 b	24.08 ab
	Control	RUI	1.63 a	0.28 a	0.49 b	0.21 b	609.63 a	381.06 c	368.29 c	22.37 b
		Noninoculated	0.74 c	0.24 a	0.22 a	0.12 a	569.20 a	3,550.47 a	3,480.78 a	42.16 a
		PsJN	0.90 a	0.25 a	0.25 a	0.11 b	514.53 a	2,296.84 b	2,690.81 b	44.36 a
		RUI	0.83 b	0.25 a	0.26 a	0.12 a	547.99 a	3,009.21 a	3,527.91 a	46.24 a
Shoot	Salinity	Noninoculated	0.71 b	0.24 a	0.34 a	0.16 a	709.96 a	4,053.75 a	4,454.38 a	44.68 a
		PsJN	0.87 a	0.17 a	0.26 b	0.12 b	485.61 a	2,485.11 b	2,837.24 b	32.71 a
		RUI	0.83 a	0.19 a	0.26 b	0.12 b	504.33 a	2,785.37 ab	3,238.48 b	38.08 a

^zPsJN treatments were inoculated with *Burkholderia phytofirmans* PsJN. RUI treatments were inoculated with *Burkholderia gladioli* RUI.

^yValues are means of four replicates. Values with the same letter within each column (noninoculated, PsJN, and RUI) indicated no significant difference based on Fisher's protected least significant difference test at $P \leq 0.05$.

97–934, 30–73, and 14–64 mg/kg for Fe, Al, and Mn, respectively, in perennial ryegrass (Mills et al., 1996). In this study, plants inoculated with ACC-deaminase bacteria had accumulated lower amounts of Fe, Mn, Zn, and Al, suggesting that they may have beneficial roles for plant growth under salinity stress by lowering the potential toxic effects of those micronutrients.

In summary, this study first reported the positive effects of ACC-deaminase-producing bacterial inoculation on the growth and salinity tolerance of perennial ryegrass widely used as a turfgrass species. The reduction in ACC accumulation in plant by the two ACC-deaminase-producing PGPB likely reduced ethylene production, which may contribute to the alleviation of salinity stress on perennial ryegrass. The ACC-deaminase-producing PGPB may be useful for turfgrass establishment and maintenance in salt-affected areas. Further efforts should be taken to examine the metabolic mechanisms or pathways of ACC-deaminase bacteria involving ethylene regulation of plant stress tolerance.

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