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Research article

The ACC deaminase expressing endophyte *Pseudomonas* spp. Enhances NaCl stress tolerance by reducing stress-related ethylene production, resulting in improved growth, photosynthetic performance, and ionic balance in tomato plants

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ABSTRACT

Plant growth promoting bacteria (PGPB) endophytes that express 1-aminocyclopropane-1-carboxylate (ACC) deaminase reportedly confer plant tolerance to abiotic stresses such as salinity by lowering stress-related ethylene levels. Two preselected ACC deaminase expressing endophytic *Pseudomonas* spp. strains, OFT2 and OFT5, were compared in terms of their potential to promote plant growth, leaf water contents, photosynthetic performance, and ionic balance of tomato plants under conditions of moderate NaCl stress (75 mM). Salinity stress strongly affected growth, leaf water contents, and photosynthetic performance of tomato seedlings, and inoculation with either OFT2 or OFT5 ameliorated these adverse effects. Decreases in plant biomass due to salinity stress were significant in both uninoculated control plants and in plants inoculated with OFT2 compared with plants without NaCl stress. However, no reductions in total biomass were observed in plants that were inoculated with the OFT5 strain. Strain OFT5 influenced growth, physiological status, and ionic balance of tomato plants more efficiently than strain OFT2 under NaCl stress. In particular, inoculated OFT5 reduced salt-induced ethylene production by tomato seedlings, and although it did not reduce shoot uptake of Na, it promoted shoot uptake of other macronutrients (P, K, and Mg) and micronutrients (Mn, Fe, Cu, and Zn). These nutrients may activate processes that alleviate the effects of salt, suggesting that OFT5 can be used to improve nutrient uptake and plant growth under moderate salt-affected conditions by reducing stress-related ethylene levels.

1. Introduction

Due to climate change, salinity is an increasing global issue, with more than 800 million hectares of land on which salinity levels may substantially reduce crop productivity (Munns and Tester, 2008). In addition, saline conditions are known to suppress plant growth under irrigation conditions, leading to substantial implications for vegetable crops (Parida and Das, 2005). Tomato (*Solanum lycopersicum* L.) is one of the most widely grown crops with an annual global production of about 50 million metric tons and tomato production has been limited by salt contents of soil and irrigation water (Turhan and Seniz, 2012). Accordingly, the consequences of salt stress have stimulated multiple studies of salt tolerance with the intention of improving plant crop yields (Win et al., 2016).

Salt induces osmotic stress by limiting absorption of water from the soil and leads to ionic stress from potentially toxic salt concentrations in plant cells (Munns and Tester, 2008). Salinity-induced reductions in plant biomass are largely the consequence of reduced leaf areas and photosynthesis, and these have been correlated with reduced leaf water potential (Win et al., 2016), which reduces stomatal conductance and eventually inhibits photosynthesis (Baker and Rosenqvist, 2004). Stomatal closure may also result in increased susceptibility to photodamage (Powles, 1984), which is predominantly avoided by increases in non-photochemical quenching energy dissipation (Weis and Berry, 1987; Krause and Weis, 1991). Accordingly, chlorophyll fluorescence parameters accurately reflect photosynthetic efficacy and energy conversion efficiency (Maxwell and Johnson, 2000). Previous reports show that chlorophyll fluorescence parameters provide good indicators of abiotic stresses, including salinity stress (Naumann et al., 2007; Mehta et al., 2010; Abdeshahian et al., 2010). Due to the high sensitivity of photosystem II (PSII) to abiotic stresses, responses and adaptations to stress are widely investigated using measurements of PSII activities (Strasser et al., 2000).

The use of plant growth promoting bacteria (PGPB) is considered an

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environment friendly approach to improving salt tolerance of agricultural crops (Dimkpa et al., 2009). In addition, many PGPBs promote plant growth by expressing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which cleaves the immediate precursor of the plant hormone ethylene to produce α-ketobutyrate and ammonia (Todorovic and Glick, 2008). ACC deaminases significantly improve plant growth and tolerance to abiotic stresses by lowering stress-related ethylene levels in plants (Matsuoka et al., 2016). Hence, PGPB that express ACC deaminases have been shown to facilitate the growth of various plants under saline conditions, including cucumber (Gamalero et al., 2010), red pepper (Siddikee et al., 2011), groundnut (Saravanakumar and Samiyappan, 2007), and tomato (Mayak et al., 2004; Ali et al., 2014) plants. However, the regulatory mechanisms of ACC deaminase expressing PGPBs on plant responses and tolerance to salinity remain poorly understood.

Previously, endophytic bacterial strains were isolated from various organic crops and were isolated according to their ability to cleave ACC (Matsuoka et al., 2016). These bacteria moderated the deleterious effects of exogenous ACC in bioassays of mungbean seedlings and lowered ethylene production, presumably rendering the plants more tolerant to salt-induced stress. In the present study, we evaluated the effects of the ACC deaminase expressing endophytic *Pseudomonas* spp. strains, OFT2 and OFT5, on the negative effects of salinity in tomato seedlings. Specifically, after inoculation of tomato seedlings with ACC expressing bacterial endophytes, we (1) investigated the effects on plant growth, photosynthetic gas exchange activities, and PSII function under conditions of salt stress, and (2) determined the ensuing shoot uptake rates of macronutrients and micronutrients to investigate the growth effects of endophytes.

2. Materials and methods

2.1. Bacteria strains

The two *Pseudomonas* spp. strains OFT2 and OFT5 were recently isolated from interior tissues of organic carrot and turnip crops, respectively, at farms in the Ibaraki Prefecture, Tsukuba, Japan (Matsuoka et al., 2016). Both strains express ACC deaminase and produce indole acetic acid (IAA) and IAA-like molecules, and can reduce (E)-2-hexenal and the other green leaf volatiles and terpenoids in carrot leaves following inoculation of seeds (Matsuoka et al., 2016). In preliminary tests, both strains remained viable in the presence of up to 137 mM NaCl in phosphate buffered saline for 12 weeks.

2.2. Plant materials and growing conditions

Seeds of the *S. lycopersicum* tomato variety Momotaro were purchased from Takii Seeds & Co., Ltd., Japan and were surface sterilized using 1 min treatments with 80% ethanol followed by 5 min treatments with 2% sodium hypochlorite (NaOCl) and five washes with sterile milli-Q water. Seeds were then sown in Murashige-Skoog (MS) media at 28 °C for 8 days. The *Pseudomonas* spp. strains, OFT2 and OFT5, were grown in tryptic soy broth at 28 °C in the dark with constant shaking at 150 rpm for 24 h. Overnight cultures were then centrifuged at 10 000 rpm for 10 min at 6 °C and were then washed twice in sterile milli-Q water and diluted to an optical density of 0.4 at 600 nm, corresponding to approximately 10^7 cells mL^{-1}. After transplanting, 7-day-old seedlings were soaked for 2 h in 100 mL bacterial suspensions or in sterile milli-Q water. Two uniform seedlings were then transferred to 3 L plastic pots of 20 cm in diameter with 3000 g of commercial horticultural soil (GENKIKUN, Katakura & Co-op Agri Corporation) containing 260 mg of nitrogen, 3900 mg of phosphorus, and 200 mg of potassium per 1000 g. Plants were then grown in a temperature adjusted greenhouse at an air temperature of 28 °C/20 °C (day/night) under natural light conditions in November 2016. Pots were watered every other day with tap water, and one week after transplanting plants were re-inoculated by spreading the bacterial cultures described above (1 mL per pot, A600 nm = 0.4) on the soil around the plants. Pot positions were randomly changed daily to minimize positional effects in the growth chamber.

2.3. NaCl treatments

Three weeks after transplanting, plants were irrigated with 500 mL aliquots of tap water (0 mM NaCl) or 75 mM NaCl solution per pot and were grown for up to 21 days. To avoid contamination of plant tissues, water and NaCl solutions were introduced with a soft pour from the edge of the pot. Plant densities and sizes were sufficient for mutual shading by other plants.

2.4. Measurements of growth parameters

Leaf areas were measured using a leaf area meter (AAM-8; Hayashi Denko Co., Tokyo, Japan), and leaves, shoots, and roots were then oven dried at 80 °C for 48 h and dry weights were determined at 21 days after treatment (DAT).

2.5. Leaf relative water contents

Leaf relative water contents (RWCs) were determined at 10 and 17 DAT in 127 mm diameter disks that were cut from the third-from-top trifoliate leaves. Fresh masses (Mf) of disks were immediately recorded and the disks were then placed in closed glass vials with distilled water and incubated for 5 h in the dark at 4 °C. Samples were then removed from the water, were lightly patted dry using Kimwipes, and were immediately weighed to record fully turgid mass (Mt) values. Dry mass (Md) measurements were performed after oven drying for 48 h at 70 °C, and RWCs were determined using the following equation (Filella et al., 1998):

$$RWC\,(\%) = \frac{\text{Fresh mass (Mf)} - \text{Dry mass (Md)}}{\text{fully turgid mass (Mt)} - \text{Dry mass (Md)}} x\ 100$$

2.6. Chlorophyll contents, gas exchange, and chlorophyll fluorescence

Prior to gas exchange measurements, relative chlorophyll contents of the uppermost fully expanded leaves were measured using a soil plant analysis development analyzer (Minolta, Tokyo, Japan), which measures the transmission of wavelengths absorbed by chlorophylls in intact leaves (mid position). Determinations were performed 30 times in all replicates, and mean values were calculated for statistical analyses.

Net photosynthetic rates (Pn), stomatal conductance (gs), and transpiration rates (E) were determined between 08:30 and 11:30 in the uppermost fully expanded leaves using a portable open flow gas exchange system (LI-6400; Li-Cor, Lincoln, NE, USA) in the greenhouse. Light intensities in the assimilation chamber were set to 1000 μmol $m^{-2}s^{-1}$ for all measurements, and the leaf air vapor pressure difference was 1–2 kPa and the CO_2 concentration was 400 μmol^{-1}.

Chlorophyll fluorescence parameters were determined using a photosynthesis yield analyzer (MINI PAM, Walz, Effeltrich, Germany). In these experiments, leaves that were previously selected for measurements of photosynthetic activity were used for fluorescence measurements. Initially, leaves were dark adapted for 20 min to determine *Fv/Fm* values and variable fluorescence (*Fv*) was then calculated as the difference between *Fm* and *Fo*. Maximum efficiencies of PSII photochemistry at given light intensities (Fv'/Fm') and non-photochemical quenching coefficients (qN) were estimated accordingly to the methods reported by Baker and Rosenqvist (2004).