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Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17

Muhammad Naveed[a], Birgit Mitter[a], Thomas G. Reichenauer[b], Krzysztof Wieczorek[c], Angela Sessitsch[a,*]

[a] AIT Austrian Institute of Technology GmbH, Bioresources Unit, Konrad-Lorenz-Strasse 24, 3430 Tulln, Austria
[b] AIT Austrian Institute of Technology GmbH, Environmental Resources and Technology, Konrad-Lorenz-Strasse 24, 3430 Tulln, Austria
[c] Division of Plant Protection, Department of Crop Sciences, University of Natural Resources and Applied Sciences, Konrad-Lorenz-Strasse 24, 3430 Tulln, Austria

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ABSTRACT

Drought is one of the major environmental stresses that adversely affects crop growth and productivity worldwide. The effect of inoculation of two bacterial endophytes *Burkholderia phytofirmans* strain PsJN and *Enterobacter* sp. FD17 on growth, water status and photosynthetic activity of two maize cultivars under drought stress conditions was investigated. Plants were exposed to drought stress by withholding irrigation at vegetative growth stage (45 days after planting). The inoculant strains efficiently colonized maize seedlings and were recovered from root, shoot and leaves of both irrigated and stressed plants. Drought stress had drastic effects on growth, leaf water content and photosynthesis of maize seedlings. Our results revealed that bacterial inoculation minimized the drought stress-imposed effects significantly increasing shoot biomass, root biomass, leaf area, chlorophyll content, photosynthesis, and photochemical efficiency of PSII. Similarly, bacterized seedlings showed higher leaf relative water content (30%) compared to control, whereas 43% higher leaf damage in terms of relative membrane permeability was observed in non-inoculated plants under drought stress. Strain PsJN was more efficient than FD17 in terms of influencing growth and physiological status of the seedlings under drought stress. Our data suggest that maize plants can be protected from inhibitory effects of the drought stress by the harbored bacterial endophytes, although the degree of protection depends on the type of the bacterial strain and the plant genotype.

1. Introduction

Plants face various biotic and abiotic stresses in hostile environmental conditions. Among these, drought is a major abiotic factor that adversely affects crop growth and productivity worldwide. Drought is expected to cause serious plant growth problems for more than 50% of the arable lands by 2050 (Vinocur and Altman, 2005). Global warming will increase the severity and frequency of drought in the future leading to a possible decrease in global food production. At the same time a steadily increasing human population which could hit 9 billion by 2050 demands an increase in food supplies. The situation will in future be even more severe as desertification will further increase and the current amount of annual loss of arable area may double by the end of the century because of global warming (IPCC, 2007).

Modern agro-biotechnological strategies are being tested to enhance drought stress tolerance in plants such as the generation of transgenic plants with introduced novel genes or with altered expression levels of the existing genes (Lu et al., 2013). Development of drought-tolerant varieties through genetic engineering and plant breeding, coupled with natural resource management is also a promising and effective approach to improve agricultural productivity and water use efficiency against drought and water shortage (Warren, 1998). However, the complexity of abiotic stress tolerance mechanisms makes the task of introducing new tolerant varieties very difficult (also a long drawn procedure), and genetically modified plants are not well accepted in some regions of the world (Wahid et al., 2007).

On the one hand, plants possess natural protection systems that act against different stresses, but on the other hand, they also interact with a variety of soil microorganisms that can alleviate the

* Corresponding author. Tel.: +43 50550 3509; fax: +43 50550 3666.
E-mail address: angela.sessitsch@ait.ac.at (A. Sessitsch).

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stress symptoms (Marulanda et al., 2006). Microbial communities are able to develop a range of activities that are very important in maintaining biological balance and sustainability in soil particularly under stress conditions (Kennedy and Smith, 1995; Kavamura et al., 2013). Under stress conditions, plants are more dependent on microorganisms that are able to enhance their metabolic activity to combat stress (Kavamura et al., 2013). Rhizobacteria that exert beneficial effects on plant growth and development are referred to as plant growth promoting rhizobacteria (PGPR). PGPR are beneficial native soil bacteria that colonize the rhizosphere or plant roots and result in increased plant growth and yield (Kloepper et al., 1989). PGPR are adapted to adverse conditions and may protect plants from the deleterious effects of drought stress, thus increasing crop productivity in arid or semiarid areas (Marulanda et al., 2007; Kavamura et al., 2013; Kasim et al., 2013). Several PGPR are reported to induce drought stress tolerance in some plants such as wheat, maize, sunflower, sugarcane and green gram (Sandhya et al., 2009, 2010; Moutia et al., 2010; Vardharajula et al., 2011; Saravanakumar et al., 2011; Kasim et al., 2013). Endophytic bacteria may in future be even more important than rhizosphere bacteria, because they escape competition with rhizosphere microorganisms and achieve more intimate contact with plant tissues.

Maize (*Zea mays* L.) is the third most important food crop globally in terms of sources of energy and protein in human nutrition. It is a C4 crop with a high rate of photosynthetic activity, leading to high grain and biomass yield. Climate change and the use of marginal land for crop production require the development of innovative management systems adapted to stressful environments, particularly drought stress. Annual yield losses due to drought average around 15% of potential yield (Edmeades, 2008). Climate change and population growth suggest that the production of major crops (maize, barley, wheat etc.) will move to marginal areas, mainly with water deficit (Edmeades, 2008).

We therefore evaluated the potential of two endophytic bacterial strains, *Burkholderia phytofirmans* strain PsJN and *Enterobacter* sp. FD17, for improving physiology and growth of maize under drought stress. *B. phytofirmans* PsJN is among the best studied plant growth promoting endophytes. It colonizes the rhizosphere and endosphere, and promotes growth, and enhances abiotic and biotic stress tolerance in a variety of crops and vegetables (Mitter et al., 2013). Recently, we found that *B. phytofirmans* PsJN efficiently colonizes maize plants upon seed inoculation and enhances germination, growth and flower onset (unpublished data). *Enterobacter* sp. FD17,was previously isolated from maize by Prischl et al. (2012), is able to improve germination, growth and yield of different maize cultivars under axenic and natural soil conditions (Naveed et al., 2013). Our results suggest that microbial inoculation assuaging stresses in plants can be utilized in agriculture in an environmentally friendly manner.

2. Materials and methods

2.1. GUS labeling of Enterobacter sp. FD17

The *Enterobacter* sp. FD17 was tagged with the glucuronidase A (*gusA*) gene following the protocol described by Wilson et al. (1995) and using the construct pCAM110 in which *gusA* is under control of the ptac promoter. Briefly, wild-type strain FD17 and *E. coli* (pCAM110 plasmid) was grown in 5 ml LB medium at 28 ± 1 °C until the optical density of 0.6, at λ 600 nm. One mL bacterial cells were pelleted by centrifugation (14,000 rpm, 10 min), washed three times with ice-cold distilled water, and resuspended in 100 and 1000 μL of saline buffer (0.85% NaCl). The cell suspension 100 μL of each was mixed and the mixture was spread on the selective plate and incubated overnight at 28 ± 1 °C. Bacterial colonies carrying the *gusA* marker were selected on M9 medium [11 g $Na_2HPO_4{\cdot}12H_2O$, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, 0.24 g $MgSO_4$, 11.1 mg, 1 ml Fe-EDTA solution, 1 mL trace elements solution (Alef, 1994) containing succinate, acetate and citrate (SAC), each at a concentration of 2 g, dissolved in 1 L], amended with 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (XGlcA) (100 μg mL^{-1}), isopropyl-β-D-galactopyranoside (IPTG) (100 μg mL^{-1}) and spectinomycin (100 μg mL^{-1}) (Sigma, St. Louis, MO). Then the bacteria were examined by using an optical stereomicroscope (model SZCTV; Olympus) and an optical microscope (model BH2; Olympus).

B. phytofirmans PsJN is one of the best studied bacterial endophyte so far, originally isolated from surface-sterilized *Glomus vesiculiferum*-infected onion roots, and reported for growth promotion of various horticultural crops (Frommel et al., 1991; Nowak et al., 1995).

2.1.1. Labeling stability and bacterial growth comparison

Stability of the chromosomal integration of the *gusA* marker in strain FD17 was determined by growing in LB liquid medium for over 10 generations and then plating a dilution series on LB medium with or without the appropriate antibiotic. Furthermore, the colony and cell morphologies and growth patterns of the genetic derivatives were compared to those of the FD17 wild-type strain in LB medium and M9 minimal medium with 5% glucose (Sambrook et al., 1989).

2.2. Inoculum preparation and bacterial growth

Strains FD17::*gusA*10 and PsJN::*gusA*10 (Compant et al., 2005) were cultured in 250 mL LB broth containing spectinomycin [100 μg mL^{-1}] at 28 ± 1 °C for 48 h in an orbital shaking incubator (VWR International GmbH, Austria) at 180 rpm. The optical density of the culture was measured at λ 600 nm using a spectrophotometer (Gene Quant Pro, Gemini BV, The Netherlands) and adjusted to 0.5 to obtain a uniform population of bacteria [10^8–10^9 colony forming units (CFU) mL^{-1}] for inoculation.

2.3. Plant material and growth conditions

A pot experiment was conducted in the greenhouse at the AIT campus in Tulln/Austria [altitude (174 m) and latitude (48°9′ N] to compare the effectiveness of selected bacterial strains for promoting growth and yield of maize under drought stress conditions. Maize plants were grown in agricultural field soil collected from Tulln in Lower Austria, Austria. Soil used in the pots had the following physic-chemical characteristics: sand, 32%; silt, 38%; clay, 30%; pH, 7.28; total carbon, 2.4%; total nitrogen, 0.23%; available phosphorus, 40 mg 100 g^{-1}; extractable potassium, 19 mg 100 g^{-1}.

Maize seeds were surface-sterilized with 70% ethanol (3 min), treated with 2% sodium hypochlorite (NaClO) (5 min), and followed by repeated washing with sterile distilled water (3 times for 1 min). The efficacy of surface sterilization was checked by plating shoot and root, and aliquots of the final rinse onto LB plates. Seeds were considered to be successfully sterilized when no colonies were observed on the LB plates after inoculation for 3 days at 28 ± 1 °C. Surface-disinfected seeds (cvs. Mazurka and Kaleo, DOW AgroSciences, Vertriebsges.m.b.HNeusiedl am See, Austria) were incubated in bacterial suspension [prepared as described above (10^8–10^9 CFU mL^{-1})] for 2 h. For the control, seeds were treated with sterilized LB broth. Three inoculated seeds (10^8 bacteria per seed) were sown in pots with cylindrical shape with diameter 27 cm and height 25 cm (Plastic Moram, China) containing 15 kg of soil and thinned to one plant after one week of germination. The experiment was conducted during the period of May to July 2011 in the greenhouse. The average maximum temperature was 20.6–27.6 °C (day)