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

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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

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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)

and 10.7–15.7 °C (night). Average relative humidity in the greenhouse chamber was 30%. The photoperiod in the chamber was set to a 16 h light and 8 h dark. Pots were arranged in a chamber of the greenhouse using a completely randomized design with three replications of each treatment. Recommended doses of N, P, and K fertilizers (160-100-60 kg ha^{-1}) were applied to each pot and equal amounts of tap water was applied to the pots to maintain optimal soil moisture depending on plant and soil conditions (up to 1000 mL). Drought stress was applied by stopping irrigation after 45 days of planting. After stopping irrigation plants were observed for signs of wilting. When shrinkage of leaves and stem were clearly visible plants were harvested and soil moisture content of both normal and reduced irrigated pots was determined.

2.4. Plant growth promoting trait measurement

Plants were harvested 66 days after sowing and the data of growth and physiology parameters were recorded before and after harvesting the pots.

Plant physiological parameters were recorded at midday (between 10:00 and 13:00) of both irrigated and drought-stressed pots.

2.4.1. Gaseous exchange measurement

Gaseous-exchange measurements i.e. [photosynthetic rate (net-rate of CO_2 assimilation at light saturation) (Asat)], stomatal conductance (g_s), transpiration rate (E) and vapor pressure deficit (VpdL) were measured with a Li-6400 portable photosynthesis system (Li-Cor, Inc. Lincoln, NE, USA) equipped with a CO_2 cartridge to adjust and maintain a constant CO_2 level of 400 μmol mol^{-1} air within the leaf cuvette. Gas exchange was measured from the top third, fully developed leaf of each plant at the ambient light of stressed and non-stressed plants.

2.4.2. Chlorophyll fluorescence

Maximum photochemical efficiency of photosystem II (PSII (F_v/F_m)) was calculated from chlorophyll fluorescence data using handy PEA (Hansatech Instruments Ltd. England). Leaves were dark adapted for 30 min before the measurement.

2.4.3. Leaf area and chlorophyll content

Leaf area (3rd leaf from top) of irrigated and drought stressed plants was recorded using LI-3100C Area Meter (Li-Cor, Inc., Lincoln, NE, USA). Leaf chlorophyll content was determined by using Chlorophyll Meter (SPAD 502 Plus, Minolta, Japan). Each leaf sample was measured in at least six different areas.

2.4.4. Relative water content and membrane permeability

Flag leaves were used for measuring the relative water content (RWC) and relative membrane permeability (RMP). Leaves were cut, sealed within plastic bags and transferred to laboratory. After measuring fresh weights, leaves were placed in distilled water for 24 h at 4 °C in darkness. After soaking, leaves were carefully blotted with tissue paper and fully turgid weight was measured. Dry weight was measured after oven drying the leaf samples at 72 °C for 24 h. Relative water contents were determined following the equation described by Teulat et al. (2003). $RWC(\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fully turgid weight} - \text{Dry weight}} \times 100$

For the RMP measurement, the leaves were cut into equal pieces and transferred to test tubes containing 20 ml of deionized distilled water. The test tubes were vortexed for10 s and the solution was assayed for initial electrical conductivity (EC0). These tubes were kept at 4 °C for 24 h and then assayed for EC1. The same samples were autoclaved at 121 °C for 20 min to determine EC2. Percent RMP was calculated as following the formula described by Yang et al. (1996)

$RMP(\%) = \frac{EC1 - EC0}{EC2 - EC0} \times 100$

2.4.5. Agronomic parameters measurement

Plant agronomic parameters such as shoot and root fresh weight were record after harvest. Plant biomass (above and below ground) was determined after drying the whole plants at 72 °C for 72 h.

2.5. Detection and enumeration of inoculant strains

2.5.1. Rhizosphere colonization

Rhizosphere soil was obtained by agitating roots and sampling the soil still attached to the roots after plant harvesting. For the isolation of rhizosphere bacteria, soil slurry was prepared by mixing 5 g rhizosphere soil with 15 mL of 0.85% (w/v) NaCl solution and agitation (180 rpm) for 60 min at 30 °C. After sedimentation of soil particles, serial dilutions up to 10^{-4} were plated onto selective LB medium containing spectinomycin (100 μg mL^{-1}), 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (XGlcA) (100 μg mL^{-1}), and isopropyl-β-D-galactopyranoside (IPTG) (100 μg mL^{-1}) as described by Afzal et al. (2012). Plates were incubated at 28 ± 1 °C for 3–4 days and blue colonies were counted to determine the average colonization value.

2.5.2. Endophyte colonization of root and shoot tissues

For the isolation of endophytes, 3 g of surface-sterilized roots were homogenized in 15 mL 0.85% NaCl (w/v) solution using a sterile mortar and pestle. Similarly, 5 g shoots of each treatment were homogenized in 15 mL 0.85% NaCl (w/v) solution. The homogenized material was put in sterile plastic bags and subjected to oscillation in a pulsifier (MicrogenBioproducts Ltd., UK) for 45 s at room temperature. After settling of the solid material, serial dilutions up to 10^{-3} were spread on selective LB medium. Plates were incubated at 28 ± 1 °C for 48 h and then transferred to 4 °C for three days. Blue colonies were counted on each plate. Thirty blue colonies of each treatment were randomly picked and the identity of isolates with the inoculant strain was confirmed by restriction fragment length polymorphism (RFLP) analysis of the 16S-23S rRNAintergenic spacer region (IGS) (Reiter et al., 2002). Isolates and applied inoculant strains had identical restriction patterns.

2.5.3. Microscopy of endophytic colonization by Enterobacter sp. FD17 and B. phytofirmans PsJN

Fresh plant organs (roots, fourth internodes, and fifth leaves) removed from three six plantlets inoculated with either *gusA* marked strains PsJN and FD17, or a control (LB) were collected 60 days after inoculation. Samples were prepared for microscopy analysis as described by Compant et al. (2005), with some modifications. Briefly, plant organs (stem and leaves) were incubated in staining solutions containing IPTG at 37 °C for 48 h. The samples were destained with 70% (v/v) ethanol solution to stop the reaction. The samples were immersed in ethanol at room temperature until the removal of tissues chlorophyll. Stem sections of different treatments were cut with a microtome (LeicaVT1000S; Leica, Nussloch, Germany), collected on glass slides, examined with an inverted microscope (Axiovert 200M, Zeiss, http://www.zeiss.com/) with an integrated camera (AxioCam MRc5, Zeiss, http://www.zeiss.com/).

2.6. Hydrogen peroxide (H_2O_2) localization in leaf tissues

Hydrogen peroxide (H_2O_2) generation in leaves was qualitatively detected by the diaminobenzidinetetrahydrochloride (DAB) staining method as described by Jambunathan (2010). Leaves were

collected and incubated with 1 mg mL^{-1} DAB solution pH 3.8, followed by vacuum infiltration of the leaves at nearly 100–150 mbar for 1–2 min. Leaves were then incubated in a plastic box for 5–6 h under high humidity conditions till brown precipitates were observed. The leaf chlorophyll was removed and de-stained with ethanol (96% v/v) under heating at 40 °C. Leaf sections were cut, collected on glass slides, examined with Binocular microscope (Olympus, Japan), and photographed.

2.7. Statistical analysis

Data analyses for plant growth parameters and bacterial densities were done using SPSS software package version 19 (IBM SPSS Statistics 19, USA). Comparisons between treatments were carried out by one-way analysis of variance (ANOVA). Duncan's test was applied for ANOVA after testing homogeneity of variance (Steel et al., 1997).

3. Results

3.1. Plant physiological parameters

Inoculation with endophytic strains, PsJN and FD17, significantly increased photosynthetic rate (Asat) compared to the respective control in two maize cultivars, Mazurka and Kaleo, under both irrigated and stress conditions (Table 1). Maximum response up to 75% compared to control was recorded by PsJN inoculation in case of cv. Mazurka under drought stress. Inoculation with strain FD17 gave 53% (Mazurka) and 41% (Kaleo) increase in photosynthesis of both cultivars under drought stress conditions. Minimum response – 19% increase over control – was achieved by FD17 inoculation in Kaleo under normal irrigation. PsJN inoculated plants had higher stomatal conductance upon exposure to stress; 87% increase in Mazurka and 60% in Kaleo, compared to the non-inoculated control. Also FD17 inoculation resulted in increased stomatal conductance, i.e. 44% in Mazurka compared to the non-inoculated control under drought stress (Table 1). Minimum response – 14 and 19% increase in Kaleo and Mazurka, respectively, over control – was achieved by FD17 inoculation under normal irrigation. Inoculation with strain PsJN under stress conditions increased transpiration rate up to 84% in Mazurka and 53% in Kaleo compared to the control. Similarly, FD17 treatment gave 50% (Mazurka) and 44% (Kaleo) increase in transpiration rate under drought stress compared to the control (Table 1). The data presented in Table 2 show that inoculation increased the vapor pressure deficit (VpdL) compared to control in both cultivars under stress conditions. In general, Mazurka showed minimum response to FD17 inoculation compared to control under irrigated and stress conditions. In case of Kaleo, FD17 inoculation resulted in 1.7 and 7% increase in VpdL compared to the control under irrigated and drought stress, respectively.

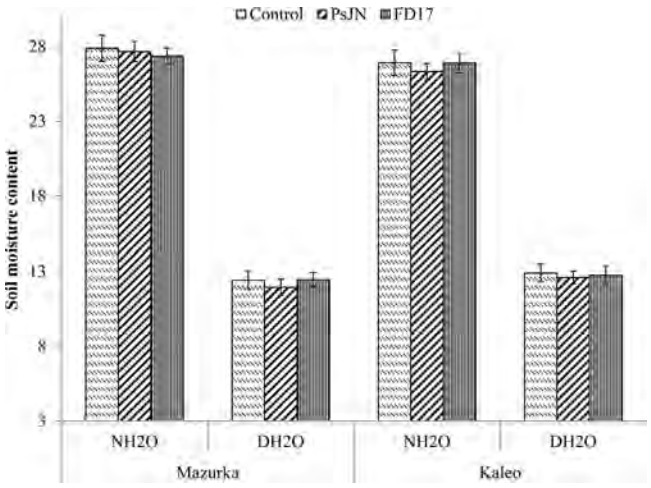

Fig. 1. Gravimetric soil moisture content (mass basis %) of normal and reduced irrigation at the time of harvest, (NH_2O; normal irrigation, DH_2O; drought stress), data are average of three replicates ± standard deviation (SD).

The maximum PSII efficiency was observed in Mazurka when inoculated with PsJN under normal condition compared to control (Table 2). PsJN inoculation increased PSII efficiency up to 10% in Mazurka compared to control under stress conditions. Kaleo showed minimum response to FD17 inoculation in regard to PSII efficiency compared to control under normal irrigation. Likewise, inoculation significantly increased chlorophyll content of both cultivars compared to control (Table 2). Inoculation with strain PsJN gave the highest increase in chlorophyll content, i.e. 22 and 19% in Mazurka and Kaleo, respectively, compared to control in both cultivars under stress. FD17 inoculation resulted in 16 and 13% increase in chlorophyll content of Mazurka and Kaleo, respectively, compared to control under the same conditions. Minimum increase – 11 and 12% in chlorophyll content of Kaleo and Mazurka, respectively over control – was achieved by FD17 inoculation under normal irrigation.

3.2. Relative water content and membrane permeability

Data in Fig. 1 shows the gravimetric soil moisture content in both normal (26–28% in Kaleo and Mazurka, respectively) and reduced irrigation (12–13% in Mazurka and Kaleo, respectively) at the time of harvest. Inoculation significantly improved RWC of both cultivars under normal and reduced irrigation (Table 2). PsJN inoculation resulted in maximum increase in RWC of Mazurka (30%) compared to control under drought stress. While 27% increase in RWC

Table 1
Effect of endophyte inoculation on physiological parameters of two maize cultivars under drought stress conditions.

Treatment	Mazurka		Kaleo		Mazurka		Kaleo	
	NH_2O[a]	DH_2O[b]	NH_2O	DH_2O	NH_2O	DH_2O	NH_2O	DH_2O
	CO_2 assimilation rate (Asat) (μmol CO_2 m^{-2} s^{-1})				Stomatal conductance (g_s) (mol H_2O m^{-2} s^{-1})			
Control	21.46 ± 2.65cd	10.87 ± 0.69g	22.80 ± 2.18c	14.77 ± 1.77f	0.120 ± 0.02bc	0.024 ± 0.01g	0.093 ± 0.01cd	0.045 ± 0.02fg
PsJN	31.11 ± 1.15a	19.06 ± 1.61de	28.49 ± 1.15ab	23.87 ± 0.84c	0.160 ± 0.01a	0.045 ± 0.01ef	0.115 ± 0.02b	0.073 ± 0.01de
FD 17	28.05 ± 1.34ab	16.63 ± 1.38ef	27.14 ± 3.95b	20.75 ± 0.83cd	0.143 ± 0.01ab	0.034 ± 0.01fg	0.106 ± 0.01c	0.062 ± 0.01ef
	Transpiration rate (E) (mmol H_2O m^{-2} s^{-1})				Vapor pressure deficit (kPa)			
Control	2.14 ± 0.50c	0.67 ± 0.09f	2.06 ± 0.42c	1.16 ± 0.39e	2.43 ± 0.08bc	2. 35 ± 0.22bc	2.32 ± 0.20bc	2.25 ± 0.09c
PsJN	3.30 ± 0.17a	1.23 ± 0.16de	2.99 ± 0.15ab	1.77 ± 0.21c	2.54 ± 0.08ab	2.72 ± 0.07a	2.45 ± 0.05bc	2.55 ± 0.02ab
FD 17	2.72 ± 0.31b	1.00 ± 0.12e	2.70 ± 0.32b	1.67 ± 0.18cd	2.47 ± 0.16bc	2.50 ± 0.11b	2.36 ± 0.16bc	2.40 ± 0.12bc

Means sharing similar letter(s) in a column for each parameter do not differ significantly at $P = 0.05$. Data are average of three replicates ± standard deviation (SD).
[a] Normal irrigation.
[b] Reduced water application.

Table 2
Effect of endophyte inoculation on relative water content, relative membrane permeability, photochemical efficiency of PSII, and chlorophyll content of two maize cultivars under drought stress conditions.

Treatment	Mazurka		Kaleo		Mazurka		Kaleo	
	NH_2O[a]	DH_2O[b]	NH_2O	DH_2O	NH_2O	DH_2O	NH_2O	DH_2O
	Relative water content (%)				Relative membrane permeability (%)			
Control	54.83 ± 3.87e	43.50 ± 1.62f	60.29 ± 1.86cd	54.96 ± 2.17e	10.52 ± 1.79b	16.85 ± 1.71a	10.20 ± 1.37b	14.60 ± 1.31a
PsJN	60.31 ± 1.09cd	56.66 ± 2.59e	74.21 ± 1.96a	69.88 ± 2.32b	6.55 ± 1.12d	9.55 ± 0.92bc	7.29 ± 1.92cd	8.62 ± 1.35bcd
FD17	61.32 ± 2.56c	55.31 ± 3.73de	70.02 ± 1.92b	64.68 ± 2.64c	6.29 ± 1.20d	9.95 ± 1.51bc	8.07 ± 1.44bcd	9.74 ± 0.80bc
	Maximum photochemical efficiency (F_v/F_m)				Chlorophyll content (spad value)			
Control	0.80 ± 0.01cd	0.74 ± 0.02e	0.80 ± 0.02cd	0.76 ± 0.03d	39.40 ± 1.45e	36.27 ± 0.40f	41.60 ± 2.30de	38.07 ± 1.05ef
PsJN	0.84 ± 0.01a	0.82 ± 0.02bc	0.83 ± 0.01ab	0.82 ± 0.02bc	46.40 ± 1.01b	44.13 ± 0.85c	48.13 ± 1.26a	45.13 ± 0.68bc
FD17	0.83 ± 0.02ab	0.81 ± 0.02c	0.82 ± 0.01bc	0.81 ± 0.02c	44.23 ± 1.49c	42.83 ± 1.04d	46.10 ± 1.31b	43.17 ± 0.86cd

Means sharing similar letter(s) in a column for each parameter do not differ significantly at $P = 0.05$. Data are average of three replicates ± standard deviation (SD).
[a] Normal irrigation.
[b] Reduced water application.

of Kaleo, was observed by PsJN inoculation compared to control under same conditions. FD17 inoculation increased RWC i.e. 27 and 20%of Mazurka and Kaleo, respectively compared to control under drought stress. The data in Table 2 show that PsJN inoculation decreased relative membrane permeability (RMP) ranges from 38 to 43% in Mazurka and 29 to 41% in Kaleo, respectively, compared to control under normal and reduced irrigation. Its maximum decrease (43%) was observed after PsJN inoculation in Mazurka under stress conditions compared to control. Inoculation with strain FD17 resulted in decrease RMP from 21% (Kaleo) to 40% (Mazurka) compared to control under normal irrigation. FD17 also decreased RMP i.e. 41% (Mazurka) and 33% (Kaleo) compared to control under drought stress.

3.3. Agronomic trait measurement

Inoculation of maize seeds with endophytic bacteria increased the number of leaves per plant, leaf area, shoot and root dry weight both under normal and reduced irrigation (Table 3). Inoculation with strain PsJN increased the number of leaves in Mazurka (24%) and Kaleo (16%), respectively, compared to control under drought stress. While FD17 resulted in a 17 and 9% increase in number of leaves of both Mazurka and Kaleo, respectively, compared to control under drought stress conditions. Likewise, inoculation increased the leaf area of both cultivars compared to non-inoculated control under normal and reduced irrigation (Table 3). PsJN inoculation increased leaf area, i.e. 21 and 20% in Kaleo and Mazurka, respectively compared to control under drought stress. FD17 resulted in 20 and 13% increase in the leaf area of both Mazurka and Kaleo, respectively, compared to control under drought stress. Data in Table 3 show that inoculation significantly increased plant biomass compared to the control. However, the inoculation response was more pronounced under water stress conditions compared to the un-inoculated control. Increased (48–66%) plant biomass was recorded in Mazurka when treated with strain PsJN compared to control under normal and stress conditions, respectively. Similarly, PsJN inoculation gave 24–46% increase in Kaleo compared to control under same conditions. FD17 inoculation resulted 42 and 32% increase in plant biomass, respectively, in Mazurka and Kaleo compared to control under drought stress. Likewise, bacterial inoculation increased root biomass of both cultivars significantly compared to control (Table 3). PsJN inoculation increased root mass, i.e. 70 and 58% in Mazurka and Kaleo, respectively, compared to control in both cultivars under stress conditions. Likewise, PsJN inoculation resulted in 47and 40% increase in Mazurka and Kaleo, respectively, compared to control under normal irrigation. FD17 inoculation increased root mass i.e.64% (Mazurka) and 34% (Kaleo), respectively, compared to control under stress conditions. The minimum response (29–36% increase) was recorded in Kaleo and Mazurka, respectively, treated with FD17 compared to control under normal irrigation.

3.4. Enumeration and microscopic localization of endophytic bacteria and ROS in plant tissues

The inoculant strains efficiently colonized rhizosphere, root and shoot interior of both maize cultivars, Mazurka and Kaleo, under normal and reduced irrigation (Fig. 2). Higher titers of PsJN (CFU g^{-1} dry weight) were recovered the rhizosphere (5.86×10^5), root interior (5.44×10^5), and shoot interior (9.36×10^4) of Mazurka under normal conditions (Figs. 2–4), and compare to Kaleo. In the case of FD17, 3.28×10^5 CFU g^{-1} DW rhizosphere, 3.06×10^5 CFU g^{-1} DW root interior and 5.97×10^4 CFU g^{-1} DW shoot interior were recorded under normal conditions. However, relatively less CFU of both strains were recorded in both cultivars under drought stress conditions. The percent viable cell numbers

Table 3
Effect of endophyte inoculation on no of leaves per plant, leaf area, shoot and root dry weight of two maize cultivars under drought stress conditions.

Treatment	Mazurka		Kaleo		Mazurka		Kaleo	
	NH_2O[a]	DH_2O[b]	NH_2O	DH_2O	NH_2O	DH_2O	NH_2O	DH_2O
	No. of leaves per plant				Leaf area (cm^2)			
Control	10.66 ± 0.54cd	9.67 ± 0.58d	11.33 ± 0.57bc	10.67 ± 0.58cd	332.60 ± 7.34c	309.94 ± 7.24d	315.77 ± 3.47d	294.10 ± 5.63e
PsJN	13.00 ± 1.00a	12.00 ± 0.43abc	13.00 ± 1.00a	12.33 ± 0.57ab	379.90 ± 6.20a	370.57 ± 6.40a	369.04 ± 5.84a	356.38 ± 7.53b
FD17	12.33 ± 1.52ab	11.33 ± 0.58bc	12.67 ± 0.57ab	11.67 ± 1.15abc	377.46 ± 9.23a	370.79 ± 6.64a	348.91 ± 3.19b	331.91 ± 6.35c
	Shoot dry matter (g)				Root dry matter (g)			
Control	24.07 ± 1.93ef	18.40 ± 1.77g	26.63 ± 1.83cd	21.70 ± 1.57f	2.49 ± 0.11d	1.41 ± 0.11f	2.46 ± 0.22d	1.55 ± 0.12f
PsJN	35.60 ± 1.93a	30.57 ± 1.66b	33.98 ± 1.87ab	31.60 ± 1.68b	3.66 ± 0.23a	2.40 ± 0.15d	3.45 ± 0.12ab	2.45 ± 0.08d
FD17	32.00 ± 1.91b	26.03 ± 1.98cd	30.70 ± 2.09b	28.67 ± 1.77c	3.38 ± 0.21bc	2.31 ± 0.10d	3.16 ± 0.15c	2.08 ± 0.09e

Means sharing similar letter(s) in a column for each parameter do not differ significantly at $P = 0.05$. Data are average of three replicates ± standard deviation (SD).
[a] Normal irrigation.
[b] Reduced water application.

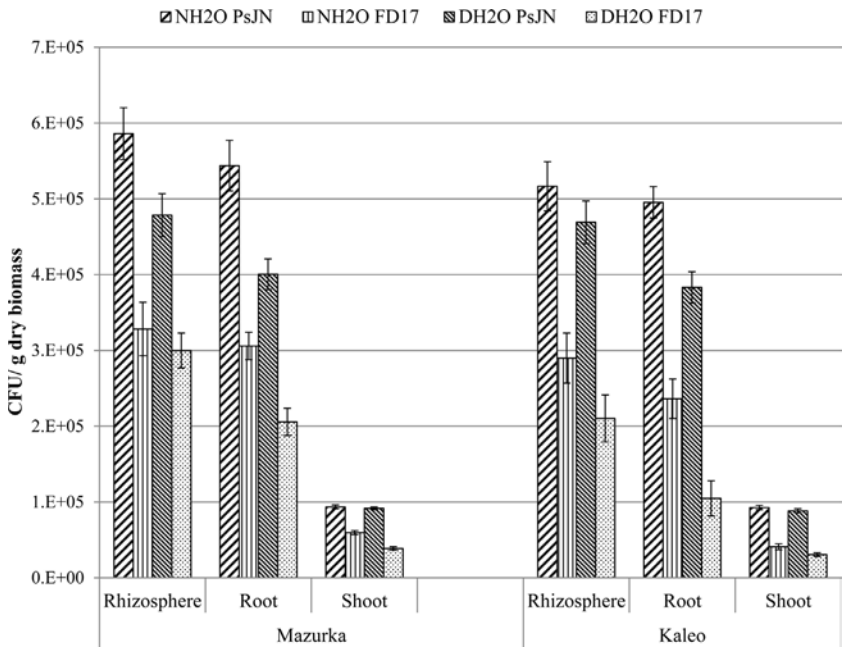

Fig. 2. Persistence of selected endophytic strains in the rhizosphere, root and shoot interior of different maize cultivars under normal and reduced irrigation (NH_2O; normal irrigation, DH_2O; drought stress), data are average of three replicates ± standard deviation (SD).

of PsJN decreased in the rhizosphere, root interior and shoot interior of Mazurka (2–26%) and Kaleo (5–23%) by drought stress. In the case of FD17, the decrease in viable cell numbers was more pronounced than with strain PsJN. Figs. 3 and 4 show the localization of the inoculated strains in different plant tissues. Fig. 5 shows the localization of H_2O_2 in the inoculated and control plants under normal and reduced irrigation. Under drought conditions more H_2O_2 was found in control than in inoculated plants.

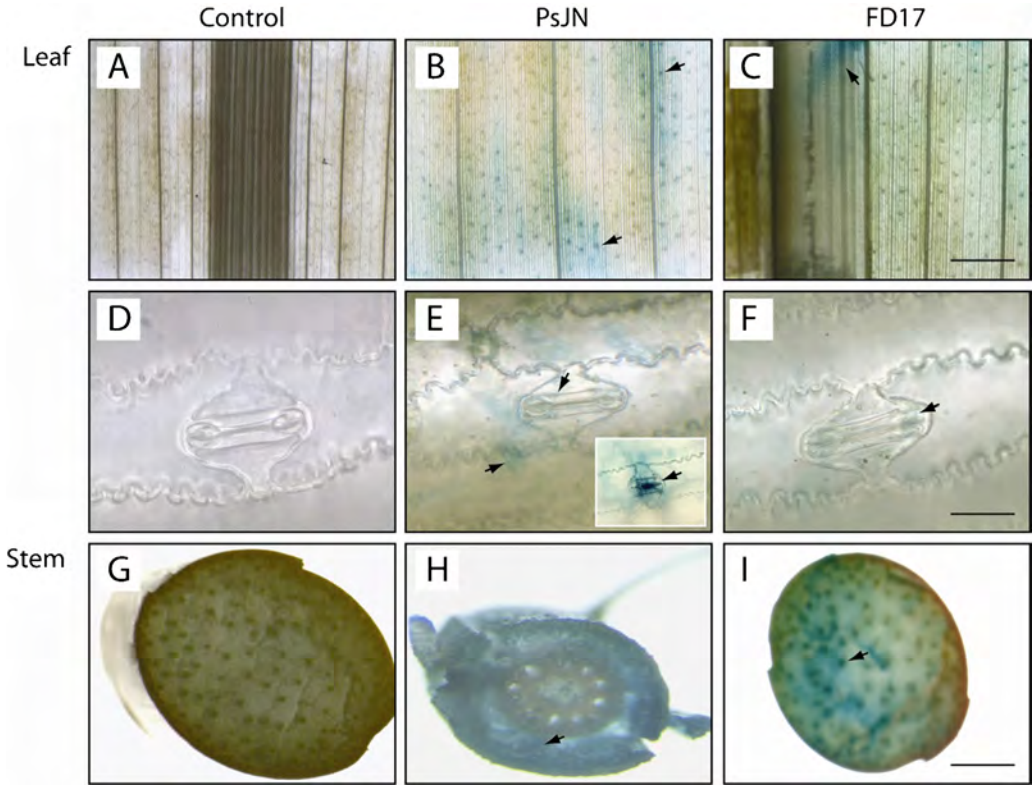

Fig. 3. Photographs of the sixth internode and leaf internal tissue of *Zea mays* L. plants inoculated with PsJN::*gusA*10 and FD17::*gusA*10. (A and G) Photographed of the sixth leaf and stem of un-inoculated control or (B and H) inoculated with PsJN::*gusA*10, and (C and I) FD17::*gusA*10 inoculated plant showing the blue color in veins due to *gusA*-marked cells (arrowheads). Inverse microscope image of the leaf stomata of un-inoculated control (D) or inoculated with (E) PsJN::*gusA*10, and (F) FD17::*gusA*10 inoculated plant, showing bacteria in the stomata and guard cell. (A–C) bars = 400 μm; (D–F) bars = 20 μm; (G–I) bars = 400 μm.

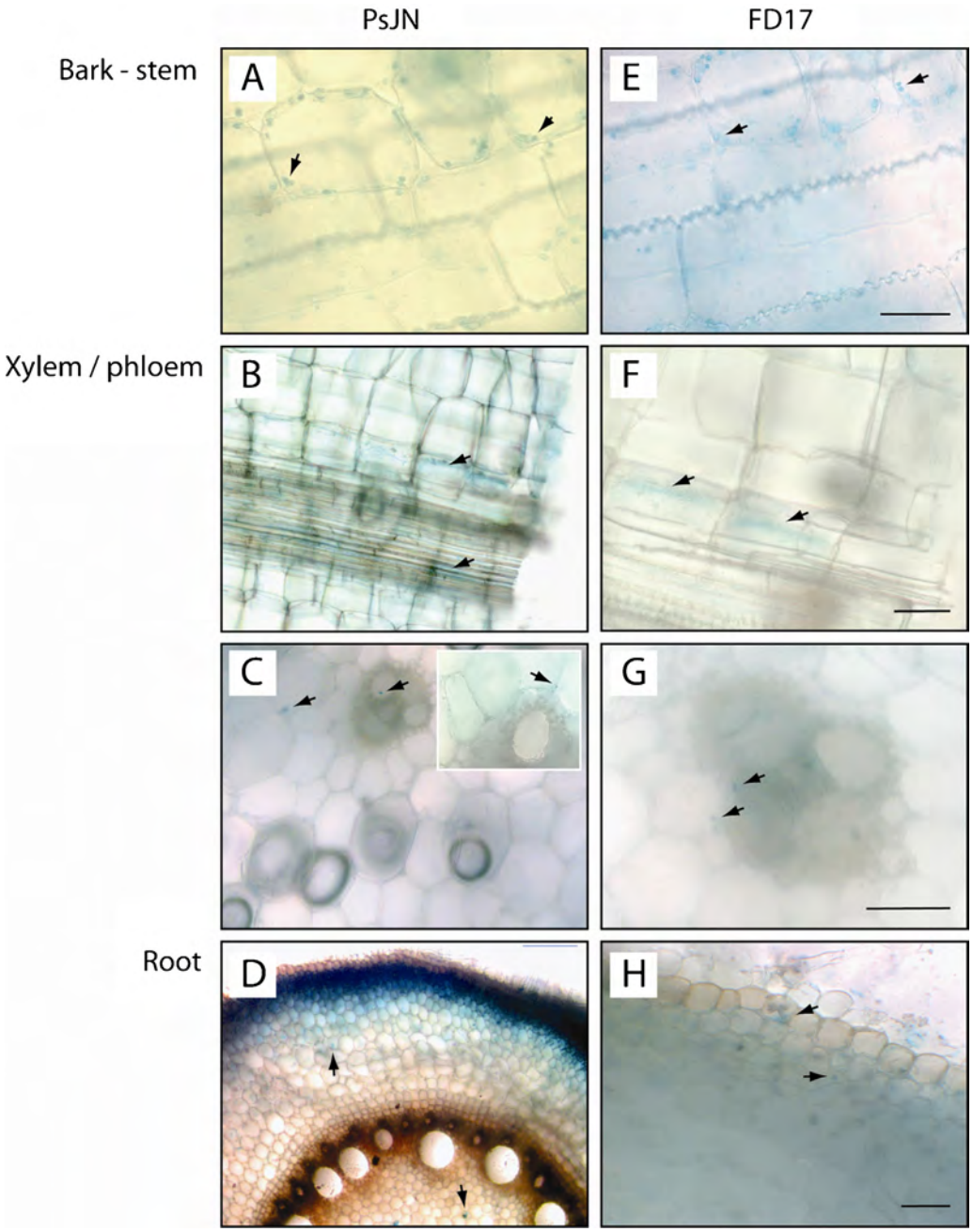

Fig. 4. Photographs of the sixth internode, leaf internal tissue and root section of PsJN::*gusA*10 and FD17::*gusA*10 inoculated *Zea mays* L. plants. (A–D) Inverse microscope image of the stem, xylem/phloem and root of PsJN::*gusA*10 inoculated plants showing blue color due to *gusA*-marked cells (arrowheads). (E–H) Inverse microscope image of the stem, xylem/phloem and root of FD17::*gusA*10 inoculated plant showing blue color due to *gusA*-marked cells (arrowheads). (A–C) bars = 20 μm; (D and H) bars = 200 μm; (E–G) bars = 20 μm.

4. Discussion

In the present study, two bacterial endophytic strains, *B. phytofirmans* PsJN and *Enterobacter* sp. FD17 were evaluated for improving growth and physiological parameters of two differentially adapted maize cultivars under drought stress conditions. PsJN colonizes the rhizosphere and endosphere, and promotes growth, and enhances abiotic and biotic stress tolerance in a variety of horticultural crops, e.g. potatoes, tomato and grapevines (Mitter et al., 2013). Very recently, Naveed et al. (2013) reported that FD17 efficiently colonizes the different cultivars of maize and enhances their growth and yield.

Endophytes live inside plants for at least part of their life cycle without being pathogenic. In contrast, some endophytes confer benefits to their plant host such as stress reduction, increased root growth and nutrient availability (Hardoim et al., 2008). Plant growth and development may be reduced in stress conditions due to impaired biochemical and physiological mechanisms. Such stresses may be relieved to some extent by the application of microbial inoculants, which evoke various natural mechanisms to help plants to sustain their growth under stress conditions (Yang et al., 2008; Vardharajula et al., 2011). In the present study, we observed that endophyte inoculation improved maize plant growth under drought stress conditions, which resulted in better survival, root/shoot biomass and water content compared to the non-inoculated control (Tables 2 and 3). Increase in the total root system is the most commonly reported plant response mediated by PGPB inoculation in various plant species (Lucy et al., 2004).

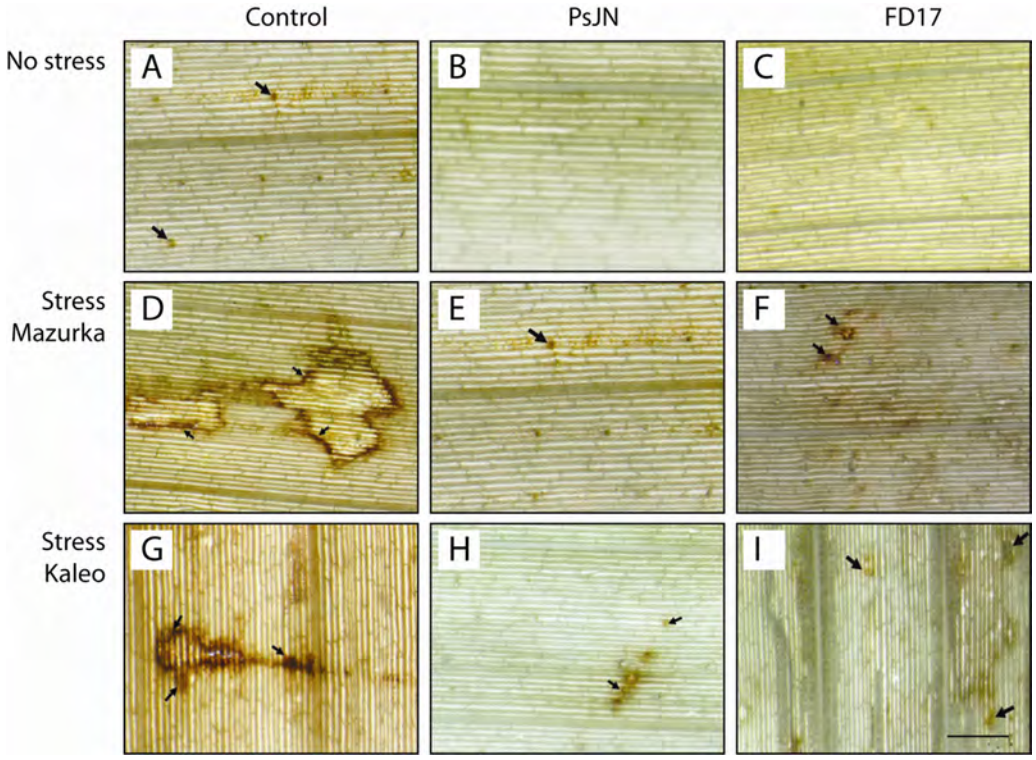

Fig. 5. Photographs of the sixth leaf internal tissue of PsJN::*gusA*10 and FD17::*gusA*10 inoculated *Zea mays* L. plants. (A, D and G) Photographed of un-inoculated control showing the ROS (H_2O_2) production under normal and drought stress (arrowheads). (B, E and H) Photographs of cv. Mazurka and Kaleo inoculated with PsJN::*gusA*10 showing H_2O_2 production under normal and drought stress (arrowheads). (C, F and I) Photographs of cv. Mazurka and Kaleo inoculated with FD17::*gusA*10 showing H_2O_2 production under normal conditions and drought stress (arrowheads). (A–I) bars = 500 μm.

This can be caused by microbial hormone production, which is considered as the most plausible mechanism in controlling root growth and development. Firstly, bacterial production of the hormone auxin in the root zone using tryptophan as a precursor from root exudates is responsible for root architecture. Bacterial-induced alterations in root architecture might lead to an increase in total root surface area, consequently improved nutrient and water uptake, which may have positive effects on plant growth as a whole (Somers et al., 2004). Secondly, under drought stress conditions, the plant hormone ethylene endogenously regulates plant homeostasis and results in reduced root and shoot growth. However, degradation of the ethylene precursor ACC by bacterial ACC deaminase releases plant stress and rescues normal plant growth (Mayak et al., 2004). As both strains used in the present study produce ACC deaminase, it is likely that the stress-induced accelerated synthesis of ethylene was reduced by inoculant strains having ACC deaminase activity resulting in longer roots, which might be helpful in the uptake of relatively more water from deep soil (Reid and Renquist, 1997; Dodd et al., 2010). Mayak et al. (2004) also reported that inoculation with PGPR containing ACC deaminase confers resistance against drought stress in tomatoes and peppers.

The inoculation of maize plants with the bacterium *B. phytofirmans* PsJN resulted in higher plant biomass production, physiology and vitality in both varieties when compared to *Enterobacter* sp. FD17 and the un-inoculated control under normal and reduced irrigation. Strain PsJN improved plant biomass and photosynthetic rate of the cultivar Mazurka up to 48 and 45%, respectively, compared to the control under normal irrigation. From numerous reports, it is evident that *B. phytofirmans* PsJN is a highly efficient plant beneficial bacterium promoting growth in a wide variety of plants (Mitter et al., 2013), however, there is evidence for plant genotype specific differences in the intensity of the effects (Pillay and Nowak, 1997; Da et al., 2012). Interestingly, this also can be seen from the present data as cultivar Mazurka responded better to PsJN inoculation than cultivar Kaleo. The data of not stressed plants indicated a correlation between growth stimulation and number of viable PsJN cells in both cultivars. It is likely that bacterial ability to promote plant growth and to establish endophytic populations is very often dependent on the plant genotype (cultivar) and developmental stage. Nowak et al. (2007) assumed that plant genotype specific differences in the plant stimulating effects are due to differences in PsJN titers in highly and poorly responsive varieties.

The response of plants to water deficit has been evaluated based on genetic, biochemical, and morpho-physiological traits. Among others, the leaf gas exchange, relative water content (RWC), photochemical efficiency of PSII, chlorophyll content, and regulation of the electron transport have been used as indicators of plant stress (Golding and Johnson, 2003; Hura et al., 2007; Maccaferri et al., 2011; Bürling et al., 2013). In the present study, bacterial colonization improved physiological traits of both maize cultivars under drought stress compared to the control. PsJN inoculation improved photosynthesis (net-rate of CO_2 assimilation under light saturation) (75%), chlorophyll content (22%) and efficiency of PSII (10%) of the cultivar Mazurka compared to the control treatment. These observations are in accordance with previous reports on the potential of endophytic bacteria having multiple beneficial traits in improving plant productivity and to enhance drought tolerance in plants (Sandhya et al., 2010; Vardharajula et al., 2011). Plant-associated bacteria may also exudate osmolytes such as proline, glycine betaine, and trehalose in response to the stress, which along with other PGP attributes can possibly act synergistically with plant-produced osmolytes and stimulate the plant growth even under stressed conditions (Paul and Nair, 2008).

The relative water content is a good indicator of water stress (Fisher, 2000) and in this study, we observed that drought caused a reduction in relative water content in both inoculated and

un-inoculated plants, however, inoculation significantly increased the relative water content compared to the un-inoculated controls. This may be due to a reduction in the inhibitory effect of drought on roots and the development of a more effective root system in the inoculated plants (Dodd et al., 2010). Drought stress accelerated relative membrane permeability (RMP) in the inoculated and un-inoculated seedlings compared to normal irrigation. However, bacterial inoculation helped seedlings to maintain the RMP and reduced 43% leaf damage compared to un-inoculated seedlings under drought stress. A positive correlation between drought stress sensitivity and membrane damage (EL) were observed by Vardharajula et al. (2011) and Sandhya et al. (2010), and the bacterial inoculation reduced the membrane damage in plants stressed by drought. In addition, reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) that cause lipid peroxidation of membranes (Sgherri et al., 2000) are produced during abiotic stresses. In our study, the inoculation reduced the H_2O_2 induced damage compared to control in both cultivars under drought stress (Fig. 5). It is most probable that bacterial colonization augmented plant defense enzymes such as catalase, peroxidase, superoxide dismutase or phenolic compounds, to alleviate the oxidative damage elicited by drought.

Soil texture is the only factor affecting the moisture content at permanent wilting. The soil moisture content at the time of permanent wilting might conceivably be affected by the plant species, environmental conditions and the soil texture. Various researchers described the soil moisture relation to plant growth, and reported that moisture content at permanent wilting vary from 1% in sand to 25% in clay and even higher in soils containing much organic matter (see Kramer, 1994; Zotarelli et al., 2010). In the present study, we observed soil moisture content in the range of 12–13% of the drought stressed pots demonstrating the moisture wilting stage (Fig. 1). At harvesting time, the soil moisture content was in the range of permanent wilting point, although the plants were not dead. It is likely that some water was available to plants even though the soil was at the permanent wilting percentage.

The occurrence and activity of microbial inoculants are affected by a variety of environmental factors faced by the plant. In the present study, endophytic populations were more suppressed and viable cell numbers decreased in Mazurka than in Kaleo under stress conditions, while in the latter cultivar the viability of endophytic bacteria were affected only to a limited extent (Fig. 2). The numbers of viable bacterial cells in stressed plants of Mazurka were far below those in the cultivar Kaleo, but at the same time the relative increase in plant growth and vitality under drought was much higher. Plants undergo a number of metabolic and physiological alterations resulting in changed root exudation during stress acclimation, which may influence the performance of an inoculant strain (Bais et al., 2006).

Out of the two endophytic strains used in this study, *B. phytofirmans* PsJN performed relatively better. This may be attributed to its intensive root/shoot colonization ability (Figs. 2–4) compared to *Enterobacter* sp. FD17, which made it more competitive. Similar findings were also obtained in other studies where strains having good root/shoot colonization showed more promising results than others (Fernandez et al., 2012; Yandigeri et al., 2012).

5. Conclusions

We provide evidence that endophytic colonization of bacteria may induce better drought stress tolerance in maize. Based on our results we conclude that application of *B. phytofirmans* strain PsJN is more efficient to improve physiology, relative water content and biomass of maize under drought conditions than *Enterobacter* sp. strain FD17. The improved plant physiology ultimately leads to enhanced crop yield and quality. Thus, endophytic bacteria could be efficiently used to reduce the effects of drought stress on growth and photosynthesis of maize.

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References

Afzal, M., Yousaf, S., Reichenauer, T.G., Sessitsch, A., 2012. The inoculation method affects colonization and performance of bacterial inoculant strains in the phytoremediation of soil contaminated with diesel oil. International Journal of Phytoremediation 14, 35–47.

Alef, K., 1994. Biologische Bodensanierung-Methodenbuch. Wiley-VCH, Weinheim, Germany.

Bais, H.P., Tiffany, L.W., Laura, G.P., Simon, G., Jorge, M.V., 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology 57, 233–266.

Bürling, K., Cerovic, Z.G., Cornic, G., Ducruet, J.-M., Noga, G., Hunsche, M., 2013. Fluorescence-based sensing of drought-induced stress in the vegetative phase of four contrasting wheat genotypes. Environmental and Experimental Botany 89, 51–59.

Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clément, C., AitBarka, E., 2005. Endophytic colonization of *Vitisvinifera* L. by plant growth-promoting bacterium, *Burkholderia* sp. strain PsJN. Applied and Environmental Microbiology 71, 1685–1693.

Da, K., Nowak, J., Flinn, B., 2012. Potato cytosine methylation and gene expression changes induced by a beneficial bacterial endophyte, *Burkholderia phytofirmans* strain PsJN. Plant Physiology and Biochemistry 50, 24–34.

Dodd, I.C., Belimov, A.A., Sobeih, W.Y., Safronova, V.I., Grierson, D., Davies, W.J., 2010. Will modifying plant ethylene status improve plant productivity in water limited environments? In: New directions for a diverse planet: Proc. Int. Crop Sci. Congr., 4th, Brisbane, Australia, 26 September–1 October 2004, Available at www.cropscience.org.au/icsc2004/poster/1/3/4/510_doddicref.htm (verified 10 January 2010). Regional Inst., Gosford, NSW, Australia.

Edmeades, G.O., 2008. Drought tolerance in maize: an emerging reality. A feature. In: James, C. (Ed.), Global Status of Commercialized Biotech/GM Crops: 2008. ISAAA Brief No. 39. ISAAA, Ithaca, NY, 12 p.

Fernandez, O., Theocharis, A., Bordiec, S., Feil, R., Jasquens, L., Clement, C., Fontaine, F., Ait Barka, E., 2012. *Burkholderia phytofirmans* PsJN acclimates grapevine to cold by modulating carbohydrate metabolism. Molecular Plant-Microbe Interaction 25, 496–504.

Fisher, D.B., 2000. Long distance transport. In: Buchanan, B.B., Gruissem, W., Jones, R.L. (Eds.), Biochemistry and Molecular Biology of Plants. Am Soc Plant Biol, MD, Rockville, pp. 730–784.

Golding, A.J., Johnson, G.N., 2003. Down-regulation of linear and activation of cyclic electron transport during drought. Planta 218, 107–114.

Hardoim, P.R., Overbeek, V., Leo, S., Elsas, D.J.V., 2008. Properties of bacterial endophytes and their proposed role in plant growth. Trends in Microbiology 16, 463–471.

Hura, T., Grzesiak, S., Hura, K., Thiemt, E., Tokarz, K., Wedzones, M., 2007. Physiological and biochemical tools useful in drought-tolerance detection in genotypes of winter triticale: accumulation of ferulic acid correlates with drought tolerance. Annals of Botany 100, 767–775.

IPCC, 2007. Climate change 2007: the physical science basis. In: Solomon, S., Qin, D., Manning, M., Chen, Z.S., Marquis, F.M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, 996 p.

Jambunathan, N., 2010. Determination and detection of reactive oxygen species (ROS), lipid peroxidation, and electrolyte leakage in plants. In: Sunkar, R. (Ed.), Plant Stress Tolerance, Methods in Molecular Biology. Humana Press, Springer, New York, pp. 291–297.

Kasim, W.A., Osman, M.E., Omar, M.N., Abd El-Daim, I.A., Bejai, S., Meijer, J., 2013. Control of drought stress in wheat using plant growth-promoting bacteria. Journal of Plant Growth Regulation 32, 122–130.

Kavamura, V.N., Santos, S.N., da Silva, J.L., Parma, M.M., Ávila, L.A., Visconti, A., Zucchi, T.D., Taketani, R.G., Andreote, F.D., de Melo, I.S., 2013. Screening of Brazilian

cacti rhizobacteria for plant growth promotion under drought. Microbiological Research 168, 183–191.
Kennedy, A.C., Smith, K.L., 1995. Soil microbial diversity and the sustainability of agricultural soils. Plant Soil 170, 75–86.
Kloepper, J.W., Lifshitz, R., Zablotowicz, R.M., 1989. Free living bacterial inocula for enhancing crop productivity. Trends in Biotechnology 7, 39–44.
Kramer, P.J., 1994. Soil moisture in relation to plant growth. The Botanical Review 10, 525–559.
Lu, Y., Li, Y., Zhang, J., Xiao, Y., Yue, Y., Duan, L., Zhang, M., Li, Z., 2013. Overexpression of Arabidopsis molybdenum cofactor sulfurase gene confers drought tolerance in maize (*Zea mays* L.). PLoS ONE 8 (1), e52126, http://dx.doi.org/10.1371/journal.pone.0052126.
Lucy, M., Reed, E., Glick, B.R., 2004. Applications of free living plant growth-promoting rhizobacteria. Antonie van Leeuwenhoek 86, 1–25.
Maccaferri, M., Sanguineti, M.C., Demontis, A., El-Ahmed, A., Garcia Del Moral, L., Maalouf, F., Nachit, M., Nserallah, N., Ouabbou, H., Rhouma, S., Royo, C., Villegas, D., Tuberosa, R., 2011. Association mapping in durum wheat grown across a broad range of water regimes. Journal of Experimental Botany 62, 409–438.
Marulanda, A., Barea, J.M., Azcon, R., 2006. An indigenous drought-tolerant strain of *Glomus intraradices* associated with a native bacterium improves water transport and root development in *Retama sphaerocarpa*. Microbial Ecology 52, 670–678.
Marulanda, A., Porcel, R., Barea, J., Azcón, R., 2007. Drought tolerance and antioxidant activities in lavender plants colonized by native drought-tolerant or drought-sensitive *Glomus* species. Microbial Ecology 54, 543–552.
Mayak, S., Tirosh, T., Glick, B.R., 2004. Plant growth-promoting bacteria that confer resistance in tomato to salt stress. Plant Physiology and Biochemistry 42, 565–572.
Mitter, B., Petric, A., Shin, M.W., Chain, P.S.G., Hauberg-Lotte, L., Reinhold-Hurek, B., Nowak, J., Sessitsch, A., 2013. Comparative genome analysis of *Burkholderia phytofirmans* PsJN reveals a wide spectrum of endophytic lifestyles based on interaction strategies with host plants. Frontiers in Plant Sciences 30, http://dx.doi.org/10.3389/fpls.2013.00120.
Moutia, J.-F.Y., Saumtally, S., Spaepen, S., Vanderleyden, J., 2010. Plant growth promotion by *Azospirillum* sp. in sugarcane is influenced by genotype and drought stress. Plant and Soil 337, 233–242.
Naveed, M., Mitter, B., Yousaf, S., Pastar, M., Afzal, M., Sessitsch, A., 2013. The endophyte *Enterobacter* sp. FD17: a maize enhancer selected based on rigorous testing of plant beneficial traits and colonization characteristics. Biology and Fertility of Soils, http://dx.doi.org/10.1007/s00374-013-0854-y.
Nowak, J., Veilleux, R.E., Nowak, S., Turgeon, S., 2007. Priming for transplant stress resistance in in vitro propagation via plantlet bacterization. Acta Horticulturae 748, 65–76.
Paul, D., Nair, S., 2008. Stress adaptations in a plant growth promoting rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. Journal of Basic Microbiology 48, 378–384.
Pillay, V.K., Nowak, J., 1997. Inoculum density, temperature, and genotype effects on in vitro growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicon esculentum* L.) seedlings inoculated with a pseudomonad bacterium. Canadian Journal of Microbiology 43, 354–361.
Prischl, M., Hackl, E., Pastar, M., Pfeiffer, S., Sessitsch, A., 2012. Genetically modified Bt maize lines containing *cry3Bb1, cry1A105* or *cry1Ab2* do not affect the structure and functioning of root-associated endophyte communities. Applied Soil Ecology 54, 39–48.
Reid, J.B., Renquist, A.R., 1997. Enhanced root production as a feed-forward response to soil water deficit in field-grown tomatoes. Australian Journal of Plant Physiology 24, 685–692.
Reiter, B., Pfeifer, U., Schwab, H., Sessitsch, A., 2002. Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* subsp. *atroseptica*. Applied and Environmental Microbiology 68, 2261–2268.
Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Laboratory Press, Cold Spring Harbor, NY.
Sandhya, V., Ali, S.Z., Grover, M., Reddy, G., Venkateswarlu, B., 2009. Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. Biology and Fertility of Soils 46, 17–26.
Sandhya, V., Ali, S.Z., Grover, M., Reddy, G., Venkateswarlu, B., 2010. Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. Plant Growth Regulation 62, 21–30.
Saravanakumar, D., Kavino, M., Raguchander, T., Subbian, P., Samiyappan, R., 2011. Plant growth promoting bacteria enhance water stress resistance in green gram plants. Acta Physiologiae Plantarum 33, 203–209.
Sgherri, C.L.M., Maffei, M., Navari-Izzo, F., 2000. Antioxidative enzymes in wheat subjected to increasing water deficit and rewatering. Journal of Plant Physiology 157, 273–279.
Somers, E., Vanderleyden, J., Srinivasan, M., 2004. Rhizosphere bacterial signaling: a love parade beneath our feet. Critical Reviews in Microbiology 30, 205–240.
Steel, R.G.D., Torrie, J.H., Dicky, D.A., 1997. Principles and Procedures of Statistics: A Biometrical Approach, 3rd ed. McGraw-Hill Book Int. Co., Singapore.
Teulat, B., Zoumarou-Wallis, N., Rotter, B., Ben Salem, M., Bahri, H., This, D., 2003. QTL for relative water content in field-grown barley and their stability across Mediterranean environments. Theoretical and Applied Genetics 108, 181–188.
Vardharajula, S., Ali, S.Z., Grover, M., Reddy, G., Bandi, V., 2011. Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. Journal of Plant Interaction 6, 1–14.
Vinocur, B., Altman, A., 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Current Opinion in Biotechnology 16, 123–132.
Wahid, A., Gelani, S., Ashraf, M., Foolad, M.R., 2007. Heat tolerance in plants: an overview. Environmental and Experimental Botany 61, 199–223.
Warren, G.F., 1998. Spectacular increases in crop yields in the twentieth century. Weed Technology 12, 752–760.
Wilson, K.J., Sessitsch, A., Corbo, J.C., Giller, K.E., Akkermans, A.D.L., Jefferson, R.A., 1995. β-Glucuronidase (GUS) transposons for ecological and genetic studies of rhizobia and other gram-negative bacteria. Microbiology 141, 1691–1705.
Yandigeri, M.S., Meena, K.K., Singh, D., Malviya, N., Singh, D.P., Solanki, M.K., Yadav, A.K., Arora, D.K., 2012. Drought-tolerant endophytic actinobacteria promote growth of wheat (*Triticum aestivum*) under water stress conditions. Plant Growth Regulation 68, 411–420.
Yang, G., Rhodes, D., Joly, R.J., 1996. Effect of high temperature on membrane stability and chlorophyll fluorescence in glycinebetaine-containing maize lines. Australian Journal of Plant Physiology 23, 431–443.
Yang, J., Kloepper, J.W., Ryu, C.M., 2008. Rhizosphere bacteria help plants tolerate abiotic stress. Trends in Plant Sciences 14, 1–4.
Zotarelli, L., Dukes, M.D., Morgan, K.T., 2010. Interpretation of Soil Moisture Content to Determine Soil Field Capacity and Avoid Over-Irrigating Sandy Soils Using Soil Moisture Sensors. University of Florida Cooperation Extension Services, AE 460. http://edis.ifas.ufl.edu/ae460.