Trehalose accumulation in *Azospirillum brasilense* improves drought tolerance and biomass in maize plants

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Abstract

Bacteria of the genus *Azospirillum* increase the grain yield of several grass crops. In this work the effect of inoculating maize plants with genetically engineered *Azospirillum brasilense* for trehalose biosynthesis was determined. Transformed bacteria with a plasmid harboring a trehalose biosynthesis gene-fusion from *Saccharomyces cerevisiae* were able to grow up to 0.5 M NaCl and to accumulate trehalose, whereas wild-type *A. brasilense* did not tolerate osmotic stress or accumulate significant levels of the disaccharide. Moreover, 85% of maize plants inoculated with transformed *A. brasilense* survived drought stress, in contrast with only 55% of plants inoculated with the wild-type strain. A 73% increase in biomass of maize plants inoculated with transformed *A. brasilense* compared with inoculation with the wild-type strain was found. In addition, there was a significant increase of leaf and root length in maize plants inoculated with transformed *A. brasilense*. Therefore, inoculation of maize plants with *A. brasilense* containing higher levels of trehalose confers drought tolerance and a significant increase in leaf and root biomass. This work opens the possibility that *A. brasilense* modified with a chimeric trehalose biosynthetic gene from yeast could increase the biomass, grain yield and stress tolerance in other relevant crops.

Introduction

Plant growth-promoting rhizobacteria stimulate growth by acting directly on plant metabolism, providing substances in short supply, fixing atmospheric nitrogen, solubilizing soil phosphorus and iron, or synthesizing phytoregulators (Bashan & de-Bashan, 2005). *Azospirillum brasilense* promotes growth of many economically important grass crops including wheat and maize (Heulin *et al.*, 1989; Caballero-Mellado *et al.*, 1992; Van de Broek *et al.*, 1993; Dobbelaere *et al.*, 2001), mainly due to the accumulation and transport of indole-3-acetic acid to the plant (Umali-García *et al.*, 1980; Hartmann *et al.*, 1983). This auxin promotes plant root elongation, which makes the uptake of mineral nutrients and water more efficient (Lin *et al.*, 1983; Kapulnik *et al.*, 1985; Dobbelaere *et al.*, 2003).

Maize is among the most prominent crops for human consumption, and, together with rice and wheat, is the staple food in most countries. One of the major challenges for the 21st century will be sufficient food production, given

estimates that the human population will reach 10 billion by 2050. This means that a strong technological effort must be conducted to increase crop yield, without increasing arable land area. On the other hand, intensive agriculture generates serious environmental problems by using chemical fertilizers, plaguicides and herbicides, salinity and water shortage (Morrissey *et al.*, 2004). All these are aggravated by climate change that is severely affecting crop productivity, and therefore improving drought tolerance in maize is of great relevance.

Plants are intermittently exposed to a plethora of abiotic stress conditions such as low or high temperature, salinity and drought (Bray *et al.*, 2000). It has been acknowledged for a long time that water availability is the stress condition that causes most losses to agriculture (Boyer, 1982). Abiotic stress triggers many common responses in plants. All of them involve cell dehydration, loss of turgor and accumulation of reactive oxygen species (ROS), which affect subcellular structures and enzyme activity. Under exposure to osmotic stress, plants exhibit a broad range of molecular and

biochemical adaptive mechanisms (Bartels & Sunkar, 2005). These comprise changes in morphology and development such as root growth inhibition, and ion transport and metabolic adjustments, which involve the synthesis of compatible solutes (osmoprotectants) that counteract dehydration and ROS by lowering osmotic potential and protecting membranes, enzymes and other subcellular structures against irreversible damage, denaturation and protein aggregation without altering physiological performance.

Among the different osmoprotectants, trehalose is one of the most effective at counteracting several types of abiotic stress (Elbein *et al.*, 2003). Trehalose (α-D-glucopyranosyl-1, 1-α-D-glucopyranoside) is a nonreducing disaccharide present in a wide variety of organisms known as anhydrobionts, which include certain species of plants, fungi, bacteria and invertebrates, and that are capable of reviving in the presence of water in a few hours after being completely dehydrated for months or years (Paul *et al.*, 2008). There are five known trehalose biosynthetic pathways (Avonce *et al.*, 2006), but the best characterized and the one present in many bacteria and plants consists of the condensation of UDP-glucose and glucose-6-phosphate by means of trehalose-6-phosphate synthase (TPS) to form trehalose-6-phosphate, which is subsequently dephosphorylated to trehalose by trehalose-6-phosphate phosphatase (TPP).

In previous reports we have shown that the overexpression of *OtsA* (bacterial TPS) gene in *Rhizobium etli* increases its osmotic stress tolerance, and using it to inoculate common bean plants improves drought tolerance and grain yield (Suárez *et al.*, 2008). Also, we have demonstrated that transformation of *Arabidopsis thaliana* with a yeast bifunctional TPS–TPP enzyme confers multiple stress protection to plants, namely freeze, heat, drought and salt tolerance (Miranda *et al.*, 2007).

Here, we analyzed the effect of inoculating maize plants with genetically engineered *A. brasilense* strains that overaccumulate trehalose.

Materials and methods

Biological material

Azospirillum brasilense strain Cd (Eskew *et al.*, 1977) and maize (*Zea maize* landrace Chalqueño, Mexico) plants were used. For gene construction and plasmid mobilization to *A. brasilense*, *Escherichia coli* S17-1 and DH5α strains were used, respectively.

Growth conditions

To inoculate plants, *Azospirillum* strains were grown on nickel fluoroborate (NFb) liquid medium (Döbereiner *et al.*, 1976) supplemented with NH_4Cl (0.2 g L^{-1}) for 2 days at 30 °C at 250 r.p.m. Bacteria were harvested by centrifuga-

tion (7000 *g* for 10 min) and washed twice in 10 mM $MgSO_4 \cdot 7H_2O$. The Congo red (Sigma Chemical Co., St. Louis, MO) agar medium (Rodríguez-Cáceres, 1982) for growing *A. brasilense* was routinely used to check culture purity and count bacteria. Maize plants were grown in 3-L plastic pots containing sterilized river sand for 2 weeks and watered five times with 200 mL of sterile distilled water. All incubations were performed in a greenhouse (24 ± 4 °C, natural illumination).

Molecular techniques

The 1452-bp *ReOtsA* coding region (Suárez *et al.*, 2008) was amplified with forward primer (5′CCG*CTCGAG*<u>AAGGAA</u> AACCCC**ATG**AGCCGTCTTAT) containing XhoI restriction site (italics), Shine–Dalgarno sequence (underlined) and initiation codon (bold) and reverse primer (5′CCC*AAGCT T*AGCGCGCTAAAGAGC**CTA**TCCGTG) containing HindIII restriction site (italics) and stop codon (bold). To construct a chimeric gene (*BIF* gene) encoding the TPS–TPP bifunctional enzyme from *Saccharomyces cerevisiae*, the full-length *TPS1* gene was fused to the three-end part of *TPS2* (Miranda *et al.*, 2007). It is known that when the N-terminal 504 amino acids are removed from *TPS2*-encoded protein, the truncation allele is still fully active as the remaining 392-amino acid carboxy-terminus encompasses the homology blocks present in all TPP enzymes (Vogel *et al.*, 1998). Therefore, we fused a *TPS1* 1526-bp fragment (encoding 493 amino acids plus two linker amino acids) to a 1353-bp fragment corresponding to the carboxy end of *TPS2* (encoding 397 amino acid plus two linker amino acids) to yield a 2873-bp DNA fragment fused through the BamHI linker site, which showed a continuous ORF encoding the predicted 892-amino acid sequence after DNA sequencing (Miranda *et al.*, 2007). The 2873-bp *TPS1-TPS2* encoding the chimeric translational fusion, here designated *BIF* gene, was amplified with forward primer (5′CCG*CTCGAG*<u>AAGGAA</u>AACCCC**ATG**ACTACGGATAAC) containing XhoI restriction site (italics), Shine–Dalgarno sequence (underlined) and initiation codon (bold) and reverse primer (5′CGG*GGTACC*ATGGTGGGT**TCA**GAC) containing KpnI restriction site (italics) and stop codon (bold). The PCR program used to amplify these sequences with High Expand DNA polymerase (Roche, Indianapolis, IN) was one cycle at 94 °C for 3 min; and 30 cycles of 94 °C for 1 min; 55 °C for 1 min; and 72 °C for 1.5 min. Both genes were checked by DNA sequencing before subcloning in the broad-host-range pBBR1MCS5 vector, which allows gene expression under the control of the *lac* promoter (Kovach *et al.*, 1995). The pBBR1M∷ReOtsA construct containing *ReOtsA* (bacterial TPS from *R. etli*) was described previously (Suárez *et al.*, 2008). In the present work, the *BIF* gene subcloned in pBBR1MCS5 vector was denominated pBBR1M∷BIF. Colonies from both plasmid constructs were

selected on gentamycin $(30 \mu g \, mL^{-1})$. Plasmids were mobilized to *A. brasilense* by conjugation with *E. coli* S17-1 harboring the corresponding construct. Wild-type *A. brasilense* was selected on ampicillin $(100 \mu g \, mL^{-1})$ and selection of transformed *A. brasilense* with either of the described plasmids was selected on gentamycin as well. Standard molecular techniques for DNA digestion, plasmid isolation and bacterial transformation were used (Sambrook & Russell, 2001).

Stress tolerance tests in *A. brasilense*

Azospirillum brasilense precultures were grown at $30 \, °C$ for 1 day in Luria–Bertani liquid media supplemented with ampicillin $(100 \mu g \, mL^{-1})$ for the wild-type strain, and $30 \mu g \, mL^{-1}$ gentamycin and ampicillin for strains harboring either pBBR1M::BIF or pBBR1M::ReOtsA plasmid. Cells were spun down and grown to mid-logarithmic phase (0.5 $OD_{600 \, nm}$) in NFb liquid media with antibiotics. To determine survival following osmotic stress, serial dilutions were made and plated on Congo red agar medium with different NaCl concentrations (0.1–0.5 M) at $30 \, °C$ for 3 days. The surviving colonies were counted afterwards.

Plant inoculation and stress tolerance tests

Maize seeds were surface sterilized in 25% commercial bleach solution (Clorox®; 6% sodium hypochlorite) for 25 min, and then washed three times with distilled sterile water. Maize seeds were germinated on 0.7% water agar plates for 24 h at $29 \, °C$; thereafter, the pregerminated seeds were sown and the seedlings were inoculated on the radicle with 1 mL bacterial suspension $(1 \times 10^8 \, CFU \, mL^{-1})$ in 10 mM $MgSO_4 \cdot 7H_2O$. Inoculated seeds were set on previously watered pots with 200 mL of nutritive solution (1 mM K_2HPO_4, 1.5 mM KH_2PO_4, 3 mM NaCl, 1.5 mM $CaCl_2$, 4.5 mM $MgSO_4$, 5 mM KNO_3, 40 μM $C_6H_5FeO_7$, 40 μM H_3BO_4, 13 μM $MnSO_4 \cdot 7H_2O$, 1.2 μM $ZnSO_4$, 0.5 μM $CuSO_4$ and 0.4 μM Na_2MoO_4, pH 6.3) and were allowed to grow for 2 weeks. Thereafter, irrigation was stopped for 10 days and was re-established afterwards. Plant recovery was observed 15 days after rewatering and the relative water content (RWC) was also determined.

Isolation of *A. brasilense* from maize roots

A detached section of maize root from each plant inoculated with the different bacterial strains was rinsed in sterile water and weighed (1–2 g), before adding 10 mM $MgSO_4 \, g^{-1}$ of tissue and shaking vigorously. Decimal dilutions were prepared from resuspended bacteria and plated in Congo red media with the corresponding antibiotic. After 3 days of incubation at $30 \, °C$, the CFU were determined.

Biomass and water content analyses

To determine plant biomass, leaves were separated from roots and oven dried at $65 \, °C$ for 3 days and weighed (dry weight). RWC and soil gravimetric water content (SGWC) were determined as reported previously (Gaxiola *et al.*, 2001). Briefly, leaves were excised, and their fresh weight was determined. After floating them in deionized water at $4 \, °C$ overnight, their rehydrated weight was determined. Plant RWC was calculated by measuring (fresh weight − dry weight)/(rehydrated weight − dry weight). SGWC was determined in fully watered soil and after 10 days without watering.

Trehalose determination

For trehalose determination, *Azospirillum* strains were grown on NFb liquid medium for 1 day at $30 \, °C$. Cultures were centrifuged, washed with water and resuspended in 80% ethanol. After incubation at $85 \, °C$ during 15 min, the samples were centrifuged and the supernatant recovered. The ethanol excess was evaporated and samples were resuspended in ultrapure water before being analyzed by HPLC using a ZORBAX carbohydrate analysis column (Agilent Technologies, Santa Clara, CA) eluted with acetonitrile : water (65 : 35 v/v). Purified trehalose (Sigma Chemical Co.) was used as a standard to determine the concentration.

Statistical analysis

All experiments were repeated at least two times independently unless otherwise stated in the figure legend. The data were processed by ANOVA using Student's *t*-test followed by Duncan–Waller mean analysis.

Results

Trehalose accumulation in *A. brasilense* confers stress tolerance

To overaccumulate trehalose in *A. brasilense* we used the broad-host-range pBBR1MCS5 vector that allows gene expression under the control of the *lac* promoter (Kovach *et al.*, 1995). Two plasmids were constructed using this vector as a backbone: the first contained the *ReOtsA* gene reported previously (Suárez *et al.*, 2008). The other construct consisted of a chimeric translational fusion of TPS and TPP from *S. cerevisiae* that we have tested before in yeast and plants (Miranda *et al.*, 2007).

To determine the possible stress tolerance in *A. brasilense* transformed with trehalose biosynthesis genes, these strains together with *A. brasilense* wild type were grown with different NaCl concentrations. The bacteria with *BIF* construction or *ReOtsA* gene grew equally well up to 0.3 M

NaCl, whereas the wild-type strain could not grow at this salt concentration. At higher NaCl concentrations reaching 0.5 M, the strain harboring *BIF* gene grew better than *A. brasilense* with *ReOtsA* gene (Fig. 1a). These results demonstrate that the trehalose biosynthesis genes expressed in *A. brasilense* confer tolerance to osmotic stress, and also shows that the *BIF* gene is more efficient than *ReOtsA*.

To further substantiate that tolerance to osmotic stress of the *A. brasilense* recombinant strains is due to the presence of trehalose, the disaccharide concentration of bacteria grown in media with 0.5 M NaCl or without salt was measured. The trehalose levels detected in *A. brasilense* harboring *ReOtsA* or *BIF* constructions rose 1.5 and 2 times, respectively, when they were grown under osmotic stress (Fig. 1b), whereas without stress, the trehalose concentration was the same in both cases and increased 1.4 times in comparison with the wild type. This strain displayed basal levels of trehalose without osmotic stress and none was detected after subjecting it to stress because it was unable to grow at 0.5 M NaCl (Fig. 1b). Thus, the increase in trehalose concentration correlated with improved stress tolerance in *A. brasilense* cells.
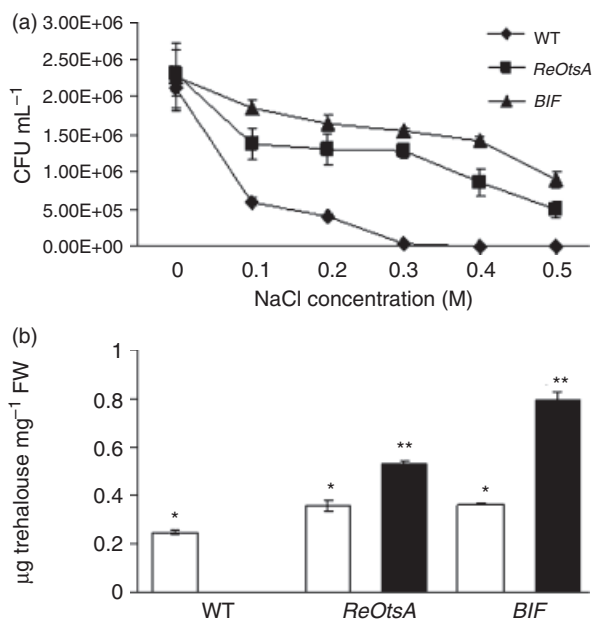
Improvement of drought tolerance in maize inoculated with *A. brasilense* containing trehalose

With the aim to determine a possible effect of *A. brasilense* recombinant strains on enhancing maize drought tolerance, plants were inoculated with *A. brasilense* harboring *ReOtsA* or *BIF* gene. Plants inoculated with *A. brasilense* wild-type strain or noninoculated plants were used as controls. Watering was stopped for 10 days in well-irrigated 2-week-old maize plants. All plants were negatively affected, with the noninoculated plants displaying the most severe wilting symptoms (Fig. 2a). At the end of the drought episode, irrigation was reestablished and plants were allowed to recover for 2 weeks. Maize plants inoculated with either of the different *A. brasilense* strains recovered much better than noninoculated plants (Fig. 2b). Plant survival was higher with those inoculated with *A. brasilense* harboring the *BIF*
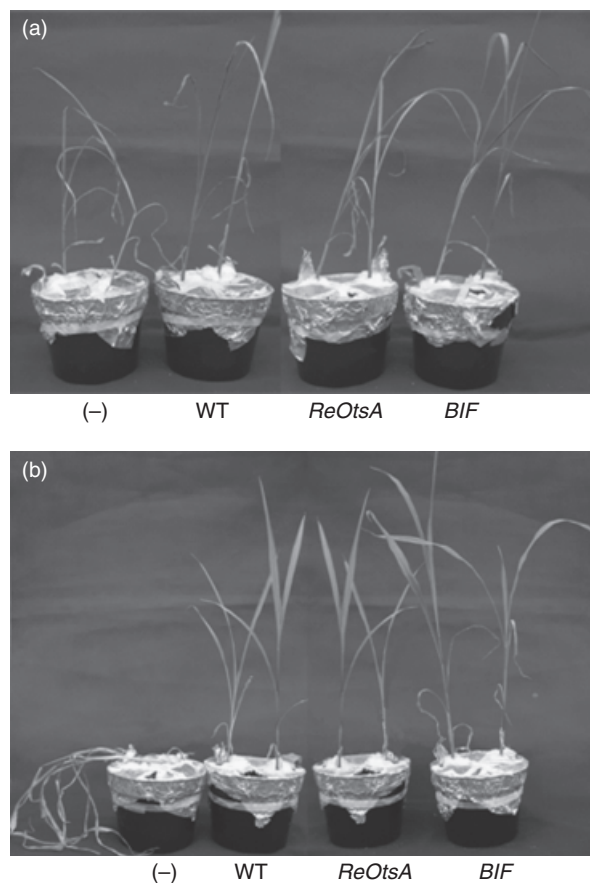

Fig. 1. Survival of recombinant *Azospirillum brasilense* upon osmotic stress. Wild type (rhombus), overexpressing *OtsA* (squares) or *BIF* gene (triangles), strains of *A. brasilense* were grown as indicated in Materials and methods (a). Wild type, overexpressing *OtsA* or *BIF* gene strains of *A. brasilense* were grown in NFb liquid without (white bars) or with 0.5 M NaCl (black bars), and aliquots were taken to measure intracellular trehalose content (b). Both figures represent the results of three independent experiments. *Means of the samples are different at the 0.05 level ($P < 0.05$). WT, wild type.

Fig. 2. Analysis of maize plants subjected to drought stress. Well-watered 2-week-old maize plants were subjected to drought stress by withholding watering for 10 days (a). After stress, plants were rewatered for 2 weeks (b). Photographs from representative plants were taken in each condition. In each pot, two plants inoculated with *Azospirillum brasilense* WT, overexpressing *ReOtsA* or *BIF* gene strains were present. WT, wild type.

gene, which displayed 85% recovery, twice that of noninoculated plants (Fig. 3a). No significant ($P < 0.05$) survival difference in drought stress tolerance was found between noninoculated plants and those inoculated with either the wild-type strain or bacteria harboring *ReOtsA*.

RWC content before drought stress shows that all plants had a very similar water status at the beginning of the
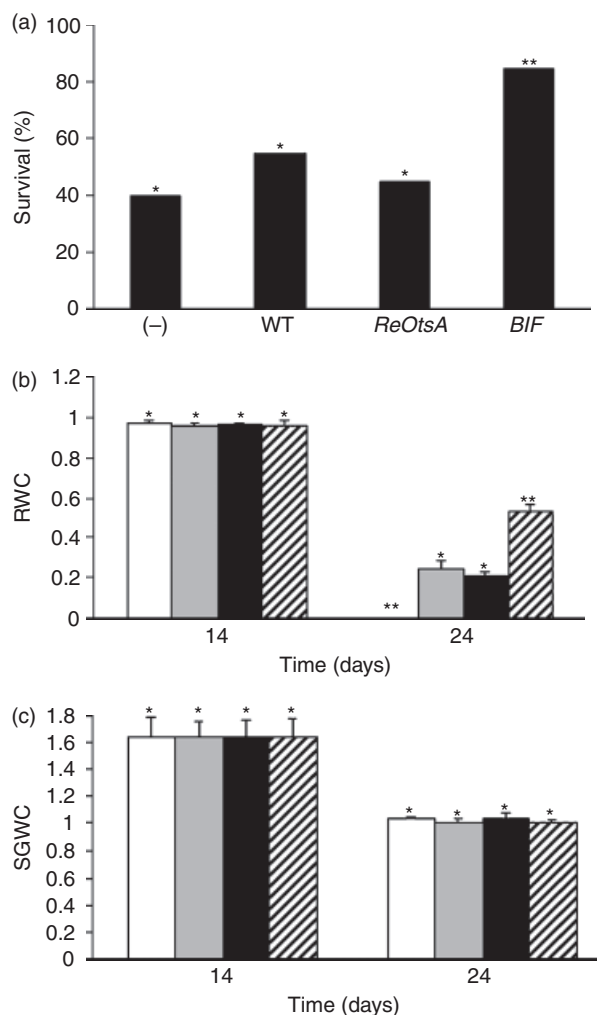

Fig. 3. Survival of plants subjected to drought stress. Well-watered 2-week-old maize plants were subjected to drought stress by withholding watering for 10 days. In each pot, two plants inoculated with *Azospirillum brasilense* WT; overexpressing *ReOtsA* or *BIF* gene strains were present. Survival to imposed drought stress on plants inoculated with either strain was determined considering 20 plants in each condition and two independent experiments were conducted. (a) Survival of plants upon continual watering was taken as 100%. (b) RWC and (c) SGWC were determined before (14 days postinoculation) and after (24 days postinoculation) water stress. In (b) and (c) noninoculated plants (white bars), or plants inoculated with *A. brasilense* WT (gray bars), overexpressing *ReOtsA* (black bars) or *BIF* gene (hatched bars), were used. *Means of the samples are different at the 0.05 level ($P < 0.05$). WT, wild type.

experiment, whereas 10 days after the stress episode, plants inoculated with *A. brasilense* containing the *BIF* gene construct retained more than twice as much water as plants inoculated with *ReOtsA* strain, or wild-type plants. After stress, practically no water was found in noninoculated maize plants (Fig. 3b). The SGWC was similar for all treatments before stress, and it dropped to 60% after the drought episode in all soil samples (Fig. 3c), indicating that the difference in plant RWC was not due to the soil water status.

Biomass increase in maize inoculated with *A. brasilense* containing trehalose

The root and aerial parts of plants inoculated with the different *A. brasilense* strains or without inoculation were weighed (Fig. 4a). Plants inoculated with *A. brasilense* harboring the *BIF* gene had a significantly increased biomass, 1.7 times greater than that of the noninoculated plants and 1.2 times (nonsignificant) greater than that of plants inoculated with *A. brasilense* wild type or harboring *ReOtsA* construct (Fig. 4a). There was no substantial increase in biomass in plants inoculated with the wild-type strain compared with noninoculated controls. These results show that there is a greater increase in maize biomass in both root
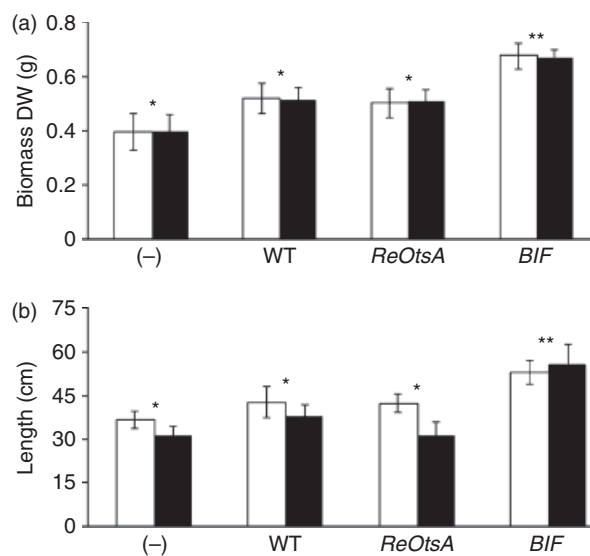

Fig. 4. Maize biomass and size determination. The foliage (white bars) and root (black bars) dry weight (DW) of maize plants after drought and recovery, at 38 days noninoculation with *Azospirillum brasilense* WT, overexpressing *ReOtsA* or *BIF* gene strains was determined (a). The aerial (white bars) and root (black bars) size in maize plants after drought and recovery, at 38 days postinoculation with *A. brasilense* WT, overexpressing *ReOtsA* or *BIF* gene strains was determined (b). Both figures represent the results of two independent experiments. *Means of the samples are different at the 0.05 level ($P < 0.05$). WT, wild type.

and foliage of *A. brasilense* expressing the *BIF* gene (Fig. 4a). Additionally, plants inoculated with *A. brasilense* containing the *BIF* gene exhibited a significantly higher plant height and root length compared with noninoculated plants or plants inoculated with the wild-type strain, or *A. brasilense* carrying the *ReOtsA* gene (Fig. 4b). These results correlate with the previously described increase in biomass using the same bacterial strain. An interesting observation is the remarkable bulky aspect of maize roots inoculated with *A. brasilense* containing the *BIF* gene compared with plants inoculated with the other strains (Fig. 5a).

To determine if trehalose was translocating from *A. brasilense* to the maize tissues, the trehalose content in maize roots and leaves was determined. No significant levels of the disaccharide could be detected in any of the analyzed tissues from plants inoculated with the different *A. brasilense* strains (data not shown).

To corroborate the presence of the different *A. brasilense* strains in the maize plants after drought stress treatment, a root sample from each plant inoculated with the different strains was taken and grown in Congo red plates with their

Fig. 5. Root morphology and bacterial persistence. Photographs from representative maize roots after drought and recovery, at 38 days postinoculation with *Azospirillum brasilense* WT, overexpressing *ReOtsA* or *BIF* gene strains (a). *Azospirillum brasilense* WT, overexpressing *ReOtsA* or *BIF* gene strains, was isolated from maize roots and counted. (b) Both figures represent the results of two independent experiments. *Means of the samples are different at the 0.05 level ($P < 0.05$). WT, wild type.

appropriate antibiotic. *Azospirillum brasilense* (WT, *ReOtsA* or *BIF*) was recovered in comparable concentrations in all cases, except for noninoculated plants, which had no bacteria attached to their roots (Fig. 5c). This clearly shows that the strains used to inoculate maize plants remained present throughout the experiment.

Discussion

Decades of breeding have had limited success in improving drought tolerance in crops due to the multigenic nature of the trait. A more promising strategy is the overexpression of genes conferring tolerance to abiotic stress. Trehalose biosynthesis has been successfully engineered in animal cells and plants to improve stress tolerance (Elbein *et al.*, 2003; Avonce *et al.*, 2004; Paul *et al.*, 2008). In the present work, we developed a novel approach to engineer stress tolerance, namely the overexpression of trehalose biosynthesis genes in *A. brasilense*, which were then used to inoculate maize plants. We used a yeast TPS–TPP bifunctional enzyme for several reasons: this chimeric construct works in distantly related species such as plants (Miranda *et al.*, 2007), and it has a strong TPP activity, thus avoiding the accumulation of free trehalose-6-phosphate in the cytosol, which is known to provoke pleiotropic effects (De Virgilio *et al.*, 1994; Schluep-mann *et al.*, 2004). Secondly, among the five trehalose biosynthetic routes, the TPS–TPP pathway is widely distributed in bacteria, fungi, invertebrates and plants, and the corresponding genes display significant identity. Thirdly, at present, there are no homolog genes available in databases from *A. brasilense* or closely related species.

First of all, we showed that *A. brasilense* has an increased tolerance to osmotic stress when it overexpressed either *ReOtsA* or *BIF* gene; however, the latter was most efficient (Fig. 1). This might be due to the fact that a bifunctional enzyme, although from a distant phylogenetically related organism such as yeast, works much better in *A. brasilense* than TPS alone to make trehalose, probably because there is a higher trehalose-6-phosphate accumulation in *A. brasilense* expressing *OtsA* compared with cells expressing the *BIF* gene. Also, higher trehalose levels in *A. brasilense* correlated with increased stress tolerance. Similarly, the overexpression of *ReOtsA* in free-living *R. etli* increased its tolerance to osmotic stress (Suárez *et al.*, 2008). In contrast, mutation of *ReOtsA* in *R. etli* impaired stress tolerance. Addition of exogenous trehalose to *Bradyrhizobium japonicum* confers tolerance to dehydration stress (Streeter, 2003). Interestingly, in other *Alphaproteobacteria* also capable of fixing atmospheric nitrogen, *Sinorhizobium meliloti* and *B. japonicum*, three different trehalose biosynthetic routes have been found (Domínguez-Ferreras *et al.*, 2006; Cytryn *et al.*, 2007). Transcription induction of their corresponding genes correlates with an increased level of trehalose, supporting an
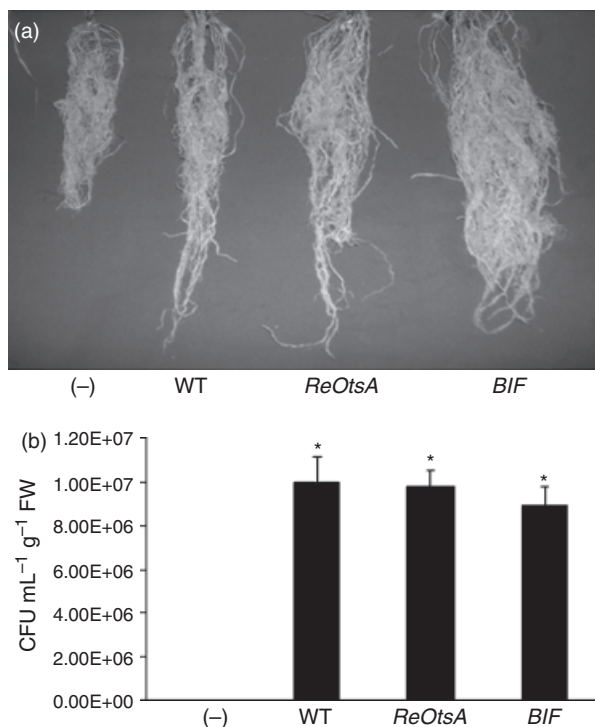
important role of this disaccharide in desiccation tolerance. A striking result is the overaccumulation of trehalose when the *A. brasilense* strains harboring *ReOtsA* or *BIF* genes were subjected to osmotic stress (Fig. 1). We have observed the same effect in *R. etli* overexpressing trehalose with the same construct harboring *ReOtsA* gene and *lacZ* promoter (Suárez *et al.*, 2008). This might be due either to the stress induction of the other trehalose biosynthetic pathways or to repression of the trehalose catabolic enzyme, or alternatively it is possible that the *lacZ* promoter used to overexpress the *BIF* gene in *R. etli* and *A. brasilense* might be stress induced.

We also examined the effects of inoculating maize plants with *A. brasilense* overexpressing either the *ReOtsA* or the *BIF* gene. Our results clearly show that the overexpression of the *BIF* gene in *A. brasilense* interacting with maize plants caused an increased drought tolerance (Figs 2 and 3). As far as we know, this is the first report of engineering drought tolerance in a grass crop by a different method than plant breeding or plant transformation. An intriguing question is whether trehalose is being transported from the bacteria to the plant, as we could not detect the disaccharide in plant tissues. We hypothesized that very small amounts of trehalose translocate to the maize roots and signal stress tolerance pathways in the plant. It is well established that trehalose plays a role as a signaling molecule (Paul *et al.*, 2008). Sugars signal growth, development and stress responses in plants and are considered to be hormone-like molecules (Rolland *et al.*, 2006). Alterations of trehalose-6-phosphate levels have demonstrated that this metabolic intermediate is indispensable for carbohydrate utilization for plant growth (Schluepmann *et al.*, 2003). Arabidopsis plants overexpressing *AtTPS1* are drought tolerant, insensitive to glucose and abscisic acid, and have shown an altered expression of *HXK1* and *ABI4* genes (Avonce *et al.*, 2004).

Another set of interesting results in the present study is a link between trehalose accumulation in *A. brasilense* and a significant increase in plant biomass. Remarkably, larger and bulky maize roots were found in plants inoculated with *A. brasilense* containing the *BIF* gene, suggesting that trehalose plays a role in root growth (Figs 4 and 5). Recently, we reported that inoculation of common bean plants with *R. etli* overexpressing trehalose caused a significant increase in biomass and grain yield (Suárez *et al.*, 2008). A transcriptomics analysis of plant nodules in symbiosis with *R. etli* overexpressing trehalose, revealed induction of several genes involved in stress tolerance, carbon and nitrogen metabolism. However, so far there is no clear evidence as to how bacterial trehalose could regulate these plant genes. Although inoculation with *A. brasilense* Cd increased grain yield through improved utilization of soil moisture (Sarig *et al.*, 1988), our present results show that the overexpression of the *BIF* gene in *A. brasilense* Cd led to an enhancement in aerial and root biomass over the wild-type Cd

strain, suggesting that it might also lead to a greater increase in maize crop yield. Therefore, *A. brasilense* engineered with the *BIF* gene is a potential tool to improve drought tolerance, biomass and possibly yield in economically important grass crops.

Acknowledgements

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