A Pseudomonas Strain Isolated from Date-Palm Rhizospheres Improves Root Growth and Promotes Root Formation in Maize Exposed to Salt and Aluminum Stress

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Abstract

The aim of this study was to evaluate the effectiveness of *Pseudomonas fluorescens 002 (P.f.002.*), isolated from the rhizosphere of date palms from the Ghardaia region in the Algerian Sahara, to promote root growth of two varieties of maize under conditions of salt and aluminum stress. Primary roots of 5-day-old seedlings were inoculated with *P.f.002.*, and seedlings were then grown under both control and stressed conditions. Primary, lateral, and seminal root lengths and numbers, as well as root dry mass, were evaluated. *P.f.002* increased all parameters measured under both salt and aluminum stress. Hence, the use of *P.f.002* may represent an important biotechnological approach to decrease the impact of salinity and acidity in crops.

Introduction

The beginning of the 21st century is marked by global scarcity of water resources, environmental pollution and increased salinization, and both excessive acidification and alkalinization of soils. The increasing human population and the reduction in land available for cultivation due to various environmental stresses (e.g. extreme temperatures, soil salinity, soil acidity, heavy metals, drought and flooding) present major threats for agricultural sustainability (Shahbaz and Ashraf, 2013).

Salinization is recognized as the main threat to agricultural resources in many countries and affects almost one billion hectares worldwide (FAO, 2008). Major factors increasing salinity, at the rate of 10% annually, include irrigation of cultivated lands with saline water, poor cultural practices, low precipitation, high temperature, and high transpiration (Shrivastava and Kumar, 2014). Soil salinity causes plant stress in two ways: (1) making water uptake by the roots more difficult, and (2) causing toxicity via accumulation of high salt concentrations in the plant (Munns and Tester, 2008). Shibli et al. (2007) observed that macronutrients N, P, K, Ca, Mg, and S decreased with elevated salinity because of an increase in Na^+ content and higher ethylene levels (Mayak et al., 2004).

Aside from salinity, Al toxicity is among the most widespread ion stresses in plants. Al has the most limiting effect on crop productivity in acid soils in tropical and subtropical regions. Dominating almost 50% of the world's cultivable area, 21% of arable lands in China, and approximately 66% of Brazil land surface; soil acidification continues to expand (Panda et al., 2009; Liu et al., 2004; Vitorello et al., 2005). Outside the tropics, enhanced Al availability has been observed as a consequence of progressive soil acidification due to air pollution from electrical power stations, industrial activities, and automobile exhaust (Smith, 1990). Root

growth inhibition is one of the earliest symptoms when plants experience Al stress, and it is observed within minutes of exposure to even micromolar concentrations of Al in solution (Rengel, 2004). Due to its ability to mimic numerous essential metals such as Fe, Ca and Mg, Al can interfere with, and cause a disruption in, a variety of biological processes such as Ca^{2+}-mediated signalling pathways, ATP stabilization, and membrane dynamics catalysed by Mg^{2+} and proteins/enzymes dependent on Fe (Mundy et al., 1997; Nayak, 2002; Perez et al., 1999).

Maize is considered to be a moderately salt- and Al-sensitive plant (Zörb et al., 2004, 2015; Fu et al., 2010; Doncheva et al., 2005). One of the strategies that have been considered to counter such environmental stressors are plant-growth-promoting rhizobacteria (PGPR) (Lutgtenberg and Kamilova, 2009; Hayat et al., 2010). These can improve plant performance under stress and, consequently, enhance yield, both directly and indirectly (Dimkpa et al., 2009). The direct mechanisms are associated with an increase in availability of nutrients and include biological nitrogen fixation, phosphate solubilization, siderophore production, and synthesis of plant hormones (Kutschera, 2007; Hayat et al., 2010). Others do this indirectly, by protecting the plant against soil-borne diseases, most of which are caused by pathogenic fungi, and other environmental stresses (Lutgtenberg and Kamilova, 2009).

Unlike in the area of salt stress, work on the effect of PGPR bacteria on aluminum (Al)-stress alleviation is not sufficiently advanced. Therefore, the purpose of this research was to study the effect of *Pseudomonas fluorescens 002* (*P.f.002*) inoculation on root growth of two maize varieties, DZ and cv. Clemente, in the presence or absence of salt and aluminum stresses.

Materials and Methods

Bacteria Strain

The bacterial strain used in this study was recently isolated. *Pseudomonas fluorescens 002* was initially isolated from the rhizosphere of date palm trees from the Ghardaia region in the Algerian Sahara. It was previously, in preliminary unpublished studies, selected on the basis of its potential as a biofertilizer (data not shown).

For inoculum preparation, bacteria were grown in King-b medium (per L: K_2HPO_4: 1.5 g, $MgSO_4.7H_2O$: 1.5 g, Pepton: 20 g, glycerol: 10 g, pH 7.2) for 24h at 28°C in a shaking incubator at 120 rpm. After 20 min of centrifugation at 20C and 4000 x g, cell pellets were harvested and resuspended with King-b medium to achieve an OD600 of 1.000.

Endogenous Indole Acetic Acid (IAA) Determination and Synthesis from Tryptophan

Bacteria were grown for the determination of endogenous IAA and potential of IAA synthesis from tryptophan according to Tsavkelova et al. (2007). Bacterial cultures were grown for 24 h in liquid media. From these bacterial inocula, 10% were transferred in 50-ml flasks to 20 ml of the same media, supplemented with 0.5 and 1.0 mmol l^{-1} of L-tryptophan (final concentration). Cultivation was performed in the dark at 28°C on a shaker (140 rpm) for 24 h. Bacterial cells were removed from cultural broth (CB) by centrifugation (2 ml of bacterial suspension). A control medium without tryptophan was used. Also a medium (zero) control with the highest tryptophan concentration has been made. The supernatant was divided into three samples as replicates. The pellet was taken up in 1 ml and also divided into three samples as replicates. The sample without addition of tryptophan indicates the endogenous IAA of the bacteria.

For free IAA determination, the supernatant of each culture was extracted for 2 h under continuous shaking after addition of 1 μg d5-IAA (Cambridge Isotope Laboratories, Andover, MA, USA) as standard to each sample. The supernatant was brought to pH 3.5 with 1N HCl and extracted twice with an equal volume of ethyl acetate. After centrifugation, the ethyl acetate fractions were combined, evaporated to dryness and resuspended in 2x 300 μl methanol. The methanol was evaporated under a stream of nitrogen, the samples resuspended in ethyl acetate for methylation. Methylation was performed by adding equal sample amounts of a 1:10 diluted solution (in diethylether) of trimethylsilyldiazomethane solution (Sigma-Aldrich) for 30 min at room temperature. The mixture was then evaporated and re-suspended in 50 mL of ethyl acetate for GC-MS analysis.

The bacterial pellet was resuspended in 1 ml methanol containing 5% acetic acid and 1μg d5-IAA was added to each sample. The samples were extracted under continuous shaking as described above. After centrifugation, the supernatant was removed, evaporated to the aqueous phase, brought to pH 3.5 and further treated as described for the culture supernatant. GC-MS analysis was carried out according to Campanella et al. (2008) on a Varian Saturn 2100 ion-trap mass spectrometer using electron impact ionization at 70 eV. The spectrometer was connected to a Varian CP-3900 gas chromatograph equipped with a CP-8400 autosampler (Varian, Darmstadt, Germany). For the analysis 1 μl of the methylated sample was injected in the splitless mode (splitter opening 1:100 after 1 min) onto a Phenomenex ZB-5 column (Aschaffenburg, Germany), 30 m · 0.25 mm · 0.25 μm, using He carrier gas at 1 ml min^{-1}. Injector temperature was 250°C, and the temperature program was 60°C for 1 min, followed by an increase of 25°C min^{-1} to 180°C, 5°C min^{-1} to 250°C, 25°C min^{-1} to 280°C, then 5 min isothermically at 280°C. Transfer line temperature was 280°C and the trap temperature was 200°C. For higher sensitivity, the μSIS mode (Varian Manual) was used. For the determination of IAA, the

quinolinium ions of the methylated substance derived from endogenous and d5-IAA at m/z 130/135 were monitored. The endogenous hormone levels were calculated by the principles of isotope dilution (Cohen et al. 1986).

From all values, the zero value (King's medium + 1 mM Trp) was subtracted. In the case of medium there was no IAA detected. Values for endogenous IAA were directly calculated as described above per ml culture or per complete bacterial pellet from a 20 ml culture. Values for the synthesis from tryptophan were calculated by subtracting the values for endogenous IAA from the values with Trp incubation.

1-Aminocyclopropane-1-Carboxylate Deaminase (ACC Deaminase) Assay

ACC deaminase was determined according to Li et al. (2011) with some modifications. The culture medium was prepared as follows: A 0.5 mol l^{-1} solution of 1-aminocyclopropane-1-carboxylate acid (ACC) was filter-sterilized through a 0.2 μm membrane, aliquoted and frozen at -20°C. Just prior to use, the frozen ACC solution was thawed and added into autoclaved medium to obtain the ACC medium with a final ACC concentration of 3.0 mmol l^{-1}. The ACC medium was diluted with the medium to respective ACC working concentrations of 0.50 and 1.0 mmol l^{-1}. The same medium was prepared as control without ACC. Bacteria were held in 5 ml of liquid medium and incubated as described above. Two ml of each culture was harvested in a 2.0 ml microcentrifuge tube by centrifugation at 8000 g for 5 min. The cell pellet was washed twice with 1 ml of liquid medium and then suspended in 2 ml of ACC and control medium in a 12 ml culture tube and incubated at 28°C on the shaker at 140 rpm for 24 h. A 2 ml sample of ACC medium without inoculation was incubated in parallel to determine the turnover of ACC in King's medium alone. The culture was centrifuged at 8000 g for 5 min. The supernatant was divided into three samples as replicates. The pellet was taken up in 1 ml

and also divided into three samples as replicates. Samples without addition of ACC represent the naturally occurring ACC in bacteria. For the zero sample, the two different ACC concentrations were placed in King's medium and extracted without incubation. This value was taken to determine the consumption of ACC in bacterial samples.

Instead of spectrophotometric determination, ACC was determined by gas chromatography-mass spectrometry (GC-MS), according to Knaust and Ludwig-Müller (2013). Briefly, the medium was diluted in methanol to yield 80% final concentration. To each sample 100 ng $[4-H_2]$-ACC (Dr. Ehrenstorfer GmbH, Augsburg, Germany) was added. Extraction was continued as described below for the cell pellet.

The cell material was homogenized under liquid N_2 using a mortar and pestle, then dissolved in 80% methanol. Extraction of ACC was carried out overnight at -20°C, then the homogenate was centrifuged at 13,000g for 15 min. The supernatant was brought to a final concentration of 80% aqueous methanol, using 100% methanol. The organic fraction was evaporated and resuspended in 60 µl acetone for derivatization with pentafluorobenzylbromide (PFBBr). Derivatization was started by adding 4 µl triethylamine and 10 µl PFBBr; the sample was incubated for 20 min at 40°C, then extracted two times with ethyl acetate. The organic phases were combined and the volume reduced to 50 µl for GC–MS analysis. The samples (1 µl) were directly analyzed because the derivatization was not stable for longer than 24 h.

The general GC-MS parameters were set as in the IAA determination. The GC-MS temperature program was set to 7 min at 150°C, then a temperature increase to 300°C, at a rate of 15°C min^{-1}. ACC-bis-PFB was analyzed in the µSIS mode (see IAA determination) using the diagnostic ions of the fragment with m/z 280 for endogenous and m/z 284 for deuterated ACC and the

levels determined using the principles of isotope dilution (Cohen et al. 1986). ACC deaminase was determined by subtracting the zero value (without incubation) from the values with ACC after incubation. The value obtained from bacteria and medium without ACC (endogenous ACC) was subtracted from this final value. Incubation of King's medium and ACC without bacteria showed that no significant amount of ACC was degraded and was therefore neglected in the calculation.

Plant Materials

Two maize varieties were studied. A local population of *Zea mays* from the Algerian Sahara (DZ) was harvested and conserved by the Laboratoire of Genetic Resources and Biotechnology of the Agricultural National High School of Algiers, and was compared to *Zea mays* cv. Clemente (KWS SAAT AG Einbeck, Germany). Seeds were surface-sterilized using a mixture of triton X-100 and 12% sodium hypochlorite for 30 min, followed by several washings with sterile distilled water. The seeds were then inoculated with bacteria cell suspension for 2h. The control seeds were treated with King-b medium for 2h.

Growth Conditions

Two independent experiments were conducted as described below for each one. 20 seeds were placed in moist filter paper for each treatment, enrolled and put vertically for germination at 25°C. Salt and aluminum treatments were applied on 5-day-old seedlings. For salt treatments, 150 mM solution was used; 8.766 g of NaCl was dissolved in 1 L of 0.5 mM $CaSO_4,2H_2O$. Aluminum treatment was prepared by adding 90 µM of $AlCl_3$ to 1 L of 0.5 mM $CaCl_2$ with a pH of 4.2. Twelve similar roots were then moved onto new filter paper watered with appropriate treatments; 150 mM NaCl, 90 µM $AlCl_3$ and distilled water for the control and maintained at 25°C for 5 days.

Growth Parameters Measurements

For the root length, principal roots were measured from the origin in the seeds to the tips. The number of seminal roots around the principal root was calculated and their average length presented the seminal roots length. The numbers of lateral roots were calculated only on the principal root. Dry mass was measured after 5 days of incubation at 50°C.

Statistical Analysis

Each treatment was analyzed with 10 - 12 replicates and data were statistically assessed by the standard deviation of the mean values and tested by Student's t-test.

Results

Endogenous Indole Acetic Acid (IAA) Determination and Synthesis from Tryptophan

IAA was determined in the pellet and the medium of *Pseudomonas fluorescens 002 (P.f. 002)* and its synthesis from tryptophan as precursor was also determined in both fractions. The data recorded for the endogenous IAA showed that *P.f.002* have the ability to secrete IAA into the medium (Table 1). After addition of tryptophan, the synthesis of IAA was marked by higher values and the amounts of IAA found in the medium enlarged with increases in the tryptophan concentration (Table 1). However in the case of the pellet, the synthesis of IAA was almost negligible in all concentrations of the assay (Table 1).

1-Aminocyclopropane-1-Carboxylate Deaminase (ACC deaminase) Assay

ACC is degraded by the enzyme ACC deaminase. The results showed that *P.f.002* can degrade ACC either in the pellet or in the medium. The degradation was observed to a much higher extent in the pellet (Table 2) compared to the King's medium without inoculation, where there no degradation was observed (Table 3).

P.f.002 Increases Primary Root Lengths under Control and Stress Conditions

The bacterium *P.f. 002* was tested for its capacity to facilitate plant growth under salt stress and aluminum (Al) toxicity. Bacterial treatments were given at the seed level, whereas seeds treated with King-b medium served as a control. *P.f. 002* increased maize growth of both maize cultivars under salt stress, Al toxicity, as well as under control conditions. With no salt or Al treatments, *P.f. 002* increased root lengths by 23% and 41% for cv. Clemente and DZ, respectively (Fig. 1A, Fig. 2A). With 150 mM NaCl, the increases of root length of cv. Cemente and DZ were by 13% and 35%, respectively (Fig. 3A, Fig. 4A). Similarly, roots inoculated with *P.f. 002* under 90 µM $AlCl_3$ were longer by 17% and 18%, for cv. Clemente and DZ, respectively (Fig. 1A, Fig 2A).

P.f.002 Stimulates Formation of Seminal Roots under Control and Stress Conditions

A 7-8% higher number of seminal roots was recorded with bacterial inoculum under control conditions of growth for cv. Clemente and DZ, respectively (Fig.1B, Fig. 2B). Under salt stress, the increases were 7% and 31% for the cultivars Clemente and DZ, respectively (Fig. 3B, Fig. 4B). The effect of *P.f. 002* was more significant under 90 µM $AlCl_3$, as the data showed an increase of 36% and 61% in seminal roots number for cv. Clemente and DZ, respectively (Fig. 1B, Fig. 2B). For the effects on the lengths of the seminal roots, the results were also impressive under NaCl and Al stress, and even under control condition. 31% and 120% increases were recorded for the control roots of cv. Clemente and DZ (without stress treatments), respectively, after *P.f. 002* inoculation (Fig. 1C, Fig. 2C).

Under salt stress, *P.f. 002* increased the development of seminal roots by 84% and 63% in length for cv. Clemente and DZ, respectively (Fig. 1C, Fig. 2C). Even more interesting results

were recorded under Al toxicity: 10.78 cm of seminal root length with bacterial inoculum compared to 2.43 cm for the control, which represents a 356% increase for cv. Clemente. Similarly, in DZ, a 208% increase in the length of seminal roots was observed; length promotion was 7.28 cm for roots with bacterial inoculum (Fig. 1C, Fig. 2C). Another parameter of growth that showed significant response to the bacterial inoculum was the number of lateral roots. Inoculation with the bacteria caused increases of 40% and 53% for the non-stressed roots, respectively, for cv. Clemente and DZ (Fig. 1D, Fig. 2D). Under salt stress, a very large difference was observed, with increases of >118% and 280% for lateral roots in DZ cv. Clemente, respectively, under the influence of bacteria (Fig. 1D, Fig. 2D). Similar results were recorded under 90 µM $AlCl_3$, where *P.f. 002* increased number of lateral roots by 161% and 116% for cv. Clemente and DZ, respectively (Fig. 1D, Fig. 2D). Similarly, of the previous parameters measured, *P.f. 002* enhanced root dry mass with or without stress. Between 19% and 29% increases were recorded for cv. Clemente and DZ, respectively, under normal conditions. The bacterial inoculum increased the dry mass more with 150 mM NaCl; results showed 45% and 66% enhancement, respectively, for cv. Clemente and DZ. Likewise, with 90 µM $AlCl_3$, the increases were 107% and 58% for cv. Clemente and DZ, respectively (Fig. 1E, Fig. 2E).

Discussion

Maize roots produce exudates that are rich in carbohydrates, sugars, and amino acids. These exuded compounds are easily available for microorganisms and may stimulate their growth (Baudoine et al., 2001), especially for those belonging to the *Pseudomonas* strains (Pandey et al., 1998). PGPR release chemicals that enhance plant growth (Sangeetha et al., 2013). Rovera et al. (2008) reported that *P. aurantiaca* SR1 mobilizes nutrients and produces indole acetic acid (IAA). Moreover, rhizobacteria release volatiles which control IAA homeostasis and cell growth in plant roots (Zhang et al., 2007). In general, rhizosphere bacteria help plants to cope with abiotic stress (Ryu et al., 2004; Yang et al., 2009; Farag et al., 2013; Nadeem et al., 2014). In our study, we

tested the potential of *Pseudomonas fluorescens 002*, based on the aforementioned characters of PGPR, to reduce the harmful effect of salt stress applied to maize seedlings. Salinity affects plant growth by imposing both ionic and osmotic stresses (Shabala and Cuin, 2008). The latter drives an increase in the Na level in the soil solution leading to a reverse movement of water out of plant cells and consequently a reduction in cell turgor pressure (Yeo et al., 1991).

In the present study, significant improvements in root length, seminal roots length, number of lateral roots and dry root mass were recorded due to inoculation with *P.f. 002* strain (Fig. 3B, Fig. 4B). El Zemrany et al. (2007) showed that the increase in the biomass of maize roots inoculated with *Azospirillum lipoferum* CRT1 was due to the higher cumulative root length and higher number of tips resulting in a significantly larger amount of branching. As a consequence, the roots had a large surface area to interact with soil particles, soil water, and microorganisms and reciprocally produced more exudates than the control (Groleau-Renaud et al., 1998). This hypothesis was confirmed by Deka Boruah et al. (2003) who showed that *Phaseolus vulgaris* roots inoculated with *Pseudomonas* strains had a higher number of lateral roots, root length, and had larger root dry mass. In saline conditions, PGPR act in different ways to stimulate plant growth. One of these mechanisms is a correction of nutrient imbalance caused by high levels of Na in soil solution. The increase in Na in soil causes an increase in Na uptake and a decrease in K and Ca content in plants (Pervaiz et al., 2002; Kholer et al., 2009). In similar conditions, Rojas-Tapias et al. (2012) reported that inoculation with *Azotobacter chroococcum* increased K, Mg and Ca concentrations and decreased Na concentration in plants. Other studies confirm these results by finding that inoculation with exopolysaccharide-producing bacteria could restrict Na influx into the roots (Ashraf et al., 2004; Kohler et al., 2006). The work of Abd El-Samad Hamdia et al. (2004) showed that maize inoculation with *Azospirillum* under salt stress increased uptake of K and Ca by restricting Na uptake. The study of Sajid et al. (2009) demonstrated that

inoculation of maize with *Pseudomonas* can permit the maintenance of a high K-Na ratio in plants, owing to lowered uptake of Na and enhanced uptake of K. Under saline stress, *Bacillus subtilis* reduces total Na uptake throughout plant tissues by regulation of high-affinity K transporters that control Na uptake (Zhang et al., 2008). Another mechanism of plant growth stimulation is the bacterial production of phytohormones and enzymes. Egamberdieva and Kucharova (2009) reported that *Pseudomonas* strains in saline condition could produce IAA, which promotes plant growth, and other lytic enzymes (lipase, cellulose, and protease) to varying degrees. *Pseudomonas* could enhance plant tolerance to salt stress by the production of the enzyme ACC deaminase, which can cleave the plant ethylene precursor ACC and thereby lower the level of ethylene in the developing or stressed plant. For many plants, a burst of ethylene is required to break seed dormancy, but, following germination, a sustained high level of ethylene may inhibit root elongation (Penrose et al., 2001). ACC deaminase has the ability to cleave ACC into ammonia and α-keto butyrate, thus reducing the effect of stress-induced ethylene on plant growth (Bochow et al., 2001; Mayak et al., 2004). The results of Nadeem et al. (2009) indicate that *Pseudomonas* strains promoted maize root growth by lowering the endogenous inhibitory levels of ethylene in roots because of their ACC deaminase activity. The study of Naz et al. (2013) showed a significant improvement in the biomass of maize roots due to a decrease in stress-induced ethylene through the ACC-deaminase activity of *Pseudomonas* strains. Moreover, auxin signaling and polar transport plays a central role in rhizobacteria-stimulated changes in the root system architecture of Arabidopsis (Zamioudis et al., 2013). Since the *Pseudomonas fluorescens* strain 002 investigated in our study was also able to synthesize IAA and reduce ACC *in vitro*, it can be assumed that part of the beneficial effect of this strain is attributable to plant hormones synthesis or modulation (Tables 1-3).

The other part of our study was the growth promotion of maize roots by a *Pseudomonas* strain under Al stress. Many reports assert the effectiveness of *Pseudomonas* on growth promotion in the absence of stress (Glick et al., 2007; Couillerot et al., 2010). However, studies are less advanced with respect to impacts of PGPR inoculation as a beneficial factor on Al toxicity. Results recorded by Lemire et al. (2010) showed that *Pseudomonas fluorescens* can survive under Al stress by orchestrating metabolic balance to counter Al toxicity. Hence, we tested the effect of the *P.f. 002* strain to alleviate damages caused by Al toxicity in maize roots. Our results were very encouraging: an increase of biomass was registered in the roots exposed for 6 days to 90 μM $AlCl_3$ solution (Fig. 3C, Fig. 4C). It is well known that roots are the most Al-sensitive organs of the plant, and that Al causes fast inhibition of the root elongation and restricts lateral root development, leading to a short stunted root system (Clarkson, 1965; Munns, 1965). Different hypotheses on the mechanisms of this Al-induced inhibition of root growth have been discussed. Al toxicity interferes with Ca metabolism (Rengel, 1992) and Al-induced inhibition of root cell division (Clarkson, 1965). Al interferes with a variety of biological processes due to its ability to mimic numerous essential metals such as Fe, Ca and Mg. In trace concentrations, this trivalent metal has been shown to interfere with protein kinase C-mediated pathways (Quarles et al., 1994), cAMP homeostasis (Hartle et al., 1996), and the glutamate-nitric oxide synthase-cGMP signalling network (Lajeunesse et al., 1998). ATP stabilization and membrane dynamics catalysed by Mg are also known to be affected by Al (Nayak, 2002). Investigations by Kidd et al. (2001) found that Al induced exudation of the flavonoid-type phenolics catechin and quercetin from 10-mm root tips in an Al-resistant maize variety. Stimulation of exudation of these flavonoid-type phenolics was in good agreement with protection of root growth against Al (Kidd et al. 2001). Enhancement of secondary metabolite production, especially of phenolics and flavonoids, may be key to enhancing maize tolerance to Al toxicity. Work conducted by Sangeetha et al. (2013) recorded that *P. fluorescens* has an efficient synthesis

of phenolic and flavonoid compounds. The study of Walker et al. (2011) revealed that the interaction between maize and PGPR caused an increase in secondary metabolite secretion by roots. Al is known to impede proteins/enzymes that are dependent on Fe to function in an effective manner due to their similar trivalent state; Al can readily substitute for Fe especially in environments where the former is easily accessible (Perez et al., 1999). Plants secrete such organic acids as citrate, malate, and oxalate to sequester Al in an effort to minimize the negative influence of this metal on Fe homeostasis (Morita et al., 2008). Another mechanism with which *Pseudomonas* can help the plant survive in Al toxicity conditions is the production of siderophores. One of the plant strategies to assimilate iron is the uptake by plants of microbial Fe^{3+} siderophores (Bienfait, 1989). The work by Dileep Kumar et al. (2001) on the potential for improving the production of pea by inoculation with PGPR showed that, in a synthetic culture medium, *Pseudomonas* strains produced siderophores. As summarized by Loper and Buyer (1991), the studies of Cody and Gross (1987a), Meyer and Abdalah (1978), and Teintze et al. (1981) also recorded that *Pseudomonas* strains are able to produce siderophores.

Conclusions

Abiotic stress conditions represent among the most important constraints on agricultural production in the world. Plant-associated microorganisms can play an important role in conferring resistance to abiotic stresses. We demonstrate that inoculation with *Pseudomonas fluorescens 002* PGPR releases IAA and protects plants against the inhibitory effects of NaCl and Al on root growth. However, extensive future studies are needed, especially to examine conditions of Al toxicity, to elucidate how bacteria mediate beneficial effects on plant growth. We argue that the use of selected PGPB may constitute important inputs to decrease the deleterious effects of saline and acidic soils.

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Figure Legends

Figure 1. Effect of NaCl, $AlCl_3$ and inoculation with *P.f. 002* on plant biomass of *Zea mays* cv. Clemente expressed as: (A) root length, (B) number of seminal roots, (C) length of seminal roots, (D) number of lateral roots and (E) root dry mass. Each value is the mean of twelve replicates. Error bars represent ± standard deviation. The asterisks indicate significant differences (* $p < 0.05$, ** $p < 0.01$) tested by t-test.

Figure 2. Effect of NaCl, $AlCl_3$ and inoculation with *P.f.* 002 on plant biomass of *Zea mays* cv. DZ expressed as: (A) root length, (B) number of seminal roots, (C) length of seminal root s, (D) number of lateral roots and (E) root dry mass. Each value is the mean of ten to twelve replicates. Error bars represent ±standard deviation. The asterisks indicate significant differences (* $p < 0.05$, ** $p < 0.01$) tested by t-test.

Figure 3. Effect of *Pseudomonas fluorescens 002* on growth and NaCl and Al tolerance of *Zea mays* cv. Clemente. (A) Representative roots treated or untreated with *Pseudomonas fluorescens 002* under no salt or Al stresses. (B) Representative roots treated or untreated with *Pseudomonas fluorescens 002* under 150 mM NaCl. (C) Representative roots treated or untreated with *Pseudomonas fluorescens 002* at 90 µM $AlCl_3$. Five days after germination, roots were exposed to: distilled water, 150 mM NaCl, and 90 µM $AlCl_3$ for (A), (B), and (C), respectively. Five days later, roots were photographed.

Figure 4. Effect of *Pseudomonas fluorescens 002* on growth and NaCl and Al tolerance of *Zea mays* cv. DZ. (A) Representative roots treated or untreated with *Pseudomonas fluorescens 002* under no NaCl or Al stresses. (B) Representative roots treated or untreated with

Pseudomonas fluorescens 002 at 150 mM NaCl. (C) Representative roots treated or untreated with *Pseudomonas fluorescens 002* at 90 µM AlCl$_3$. Five days after germination, roots were exposed to: distilled water, 150 mM NaCl, and 90 µM AlCl$_3$ for (A), (B), and (C), respectively. Five days later, roots were photographed.

Tables

Table 1: Capacity of *P.f.002* to produce endogenous IAA, and to synthesize IAA from two concentration of tryptophan, in the pellet and in the medium.

Table 2: Capacity of *P.f.002* to degrade ACC present in two concentrations, in the pellet and in the medium.

Table 3: ACC variation in King's b medium without inoculation with *P.f.002* after 24h in the presence of 1 mM ACC in solution.

Table 1

Trp conc (mM)	IAA (ng / pellet)	IAA (ng / ml medium)
0	2.65	388.98
0	4.91	519.92
0	4.28	266.83
0.5	1.62	767.73
0.5	6.33	647.48
0.5	5.46	896.91
1	4.69	1097.67
1	24.61	1051.29
1	11.81	867.28

Table 2

ACC conc (mM)	Pellet (ng / pellet)	Medium (ng / ml)
0.5	1250.14	52.97
0.5	374.2	259.99
0.5	3055.64	97.04
1	7316.88	919.74
1	4877.65	489.05
1	7552.27	28.46

Table 3

	ACC (ng)		
0h	10620.01	5797.69	9582.53
24h	9297.55	9250.61	10059.18