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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.

1. 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 pro-

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teins/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.

2. Materials and methods

2.1. Bacteria strain

The bacterial strain used in this study was recently isolated. *P. 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: K_2HPO_4: 1.5 g, $MgSO_4\,7H_2O$: 1.5 g, Pepton:20 g, glycerol: 10 g, pH 7.2) for 24 h at 28 °C in a shaking incubator at 120 rpm. After 20 min of centrifugation at 20 °C and 4000 × *g*, cell pellets were harvested and resuspended with King-b medium to achieve an OD600 of 1.000.

2.2. 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 1 N 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 2 × 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 1 μg 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.

2.3. 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.