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Enhanced symbiotic performance and productivity of drought stressed common bean after inoculation with tolerant native rhizobia in extensive fields

Youssef Yanni[a,*], Mohamed Zidan[b], Frank Dazzo[c], Rizk Rizk[a], Ahlam Mehesen[a], Faiza Abdelfattah[a], Abdelgawad Elsadany[a]

[a] Department of Microbiology, Sakha Agricultural Research Station, Kafr Elsheikh, 33717, Egypt
[b] Department of Plant Nutrition, Sakha Agricultural Research Station, Kafr Elsheikh, 33717, Egypt
[c] Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824, USA

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ABSTRACT

This study isolated and examined the performance of four selected strains of *Rhizobium* as growth enhancer inoculants of common bean (*Phaseolus vulgaris*) in saline- and drought-stressed fields located to the east and west of the Egypt Nile delta. Indigenous bean rhizobia were tested for salt tolerance by culturing and for taxonomic status by DNA analysis. Their nodulation and N_2-fixation abilities under drought stress and persistence in biofertilizer formulations were evaluated, followed by assessment of their agronomic performance with common bean in 16 salt-affected, drought-stressed fields in combination with different doses of N-fertilizer applications in 2007 and 2008. Population dynamics studies with one model strain indicated good persistence in biofertilizer preparation. Inoculation with a test strain increased plant weight in the greenhouse from 2.718 to 3.314 g and four strains increased seed yield in saline/drought-stressed fields by 2.848–3.218 t ha^{-1} during seasons 2007 and 2008, respectively. Inoculation also increased straw production, harvest indices and the agronomic fertilizer N-use efficiency. The corresponding mean seed yields in adjacent uninoculated farmers' fields exposed to the same intensity of aridification were 2.425 and 2.230 t ha^{-1}, respectively. The study shows that locally-selected strains of rhizobia can be formulated into biofertilizers that significantly enhances seed yield and the agronomic N-use efficiency while providing a nature-based alleviation of the abiotic water deficit stress of intense aridification in saline- and drought-stressed fields.

1. Introduction

Biofertilization of legumes improves soil fertility and decreases ground water pollution by avoiding excessive application of chemical fertilizers. In several regions of the Mediterranean basin, the productivity of summer legumes is frequently limited by water insufficiency and soil salinity, causing a need for importation to fulfill deficits in legume production.

Common bean (*Phaseolus vulgaris* L.) is produced worldwide as an important protein extender and substitute for animal protein (Duke, 1981; Kay, 1979). It is a warm season crop that performs best in fertile sandy-loam soils with moderate organic matter content, good internal or tile drainage, and a wide range of temperatures (between 10 and 27 °C). Inadequate nutrient supply, higher temperatures and moisture stresses during its flowering and pod setting stages result in abortion of many blossoms and developing pods, seriously affecting the crop yield. Effective nodulation by the symbiotic nitrogen-fixing bacterium, *Rhizobium*, is difficult to achieve in certain soil types and environmentally stressed conditions. Low humidity, soil salinity and high temperatures in the root zone adversely affect rhizobial survival and infection of bean roots, nodule formation, and development of the enzymatic system that participates in symbiotic N_2-fixation (Kaymakanova et al., 2008). Also stressful is the high loss of plant water by evapotranspiration due to high temperatures that intensify soil salinity.

* Corresponding author.
E-mail address: yanni244@yahoo.com (Y. Yanni).

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Previous efforts have been made in Egypt and elsewhere to increase the salt tolerance and manage growth and performance of legumes under conditions of severe salinity and drought stress (Abdel-Mawgoud et al., 2010; Ashraf and Iram, 2005; Bano et al., 2010; Beltagi et al., 2006; Bouhmouch et al., 2005; Shamseldin and Werner, 2004; Shamseldin et al., 2005; Tejera et al., 2004; Zahran, 1999). These earlier studies document the important benefits of establishing the *Rhizobium*-bean symbiosis, but lack the extensive field assessments needed to evaluate, identify and recommend high-performing biofertilizer strains that can be used with confidence to alleviate drought and salinity stresses when cultivated in various agroecosystems. Some of the studies were done *in vitro* and in greenhouses with strains that were not proven to be tolerant of high salinity or drought. Also, most field inoculation tests with N_2-fixing rhizobia were done in only one or two field locations. Most importantly, description of comprehensive field inoculation experiments is needed to address many issues that are required to formulate and implement a biofertilization technology for field management of the crop under these aridification stresses over broad agricultural landscapes. That lack of a proven biofertilizer efficacy based on extensive agronomic field assessments justified the present translational study. Its main goal was to determine if local superior strains of common bean rhizobia with saline-tolerant attributes can be isolated and exploited as biofertilizer inoculants that significantly improve production of common bean in regions where cultivation of this crop is severely affected by drought and salinity.

2. Materials and methods

2.1. Lab and greenhouse tests

2.1.1. Plant cultivar

According to the Egypt legislation, approval of a crop variety for use in field testing and later recommended for large scale production is only given after passing obligatory tests. Giza 6 used in this study is an improved variety that produces a white seed of medium size (41 g seed $100\,g^{-1}$) and was developed by institutions belonging to the Agricultural Research Center, Egypt (http://www.researchgate.net/institution/Agricultural_Research_Center_Egypt). The Field Crops Research Institute (http://www.fcri-egypt.org/AboutFCRI.htm) performed extensive breeding programs for its adaptation to Egypt agro-climatic factors. Tests for its resistance to fungal and viral diseases were performed by the Plant Pathology Research Institute (http://plantpathology-egypt.tripod.com/), insect pest control (Van Emden et al., 1988) by the Plant Protection Research Institute (http://www.arc.sci.eg/InstsLabs/Default.aspx?OrgID=5&lang=en), and its tolerance to soil physical and chemical stresses (mainly drought and soil salinity) by the Soils, Water and Environment Research Institute (http://www.sweri-eg.com/). The later institution selected this variety for this study due to its full capability of seed production with no evidence of salinity stress as long as adequate chemical fertilizer N was applied, or alternatively and preferably, low N-application doses plus successful biofertilization with strains that can express high efficiency in nodulation and N_2-fixation under different agronomic stresses in the cultivation area. Results of these tests indicated that this variety exhibits less potential of seed production in non-inoculated fields even when adequate fertilizer N was applied.

2.1.2. Rhizobium culture collection

Root nodules were obtained from Giza 6 common bean grown in fields expressing saline- and drought-stresses due to a shortage of irrigation water and located east and west of the Nile delta. The nodules were gently washed in running water, surface-sterilized by 70% ethanol for 3 min followed by 10% sodium hypochlorite for 1.5 min, then rolled over yeast extract mannitol agar (YEMA) plates [components ($g\,L^{-1}$): yeast extract 1.00, mannitol 10.00, K_2HPO_4 0.50, Mg SO_4 0.20, NaCl 0.10, $CaCO_3$ 1.00, Agar 15.00] to verify the efficiency of the surface-sterilization process, and macerated in sterile 5 mM Na-phosphate buffer (pH 7.0). The macerates were streaked on YEMA plates and incubated at $29 \pm 0.5\,^{\circ}C$ for 72 h. Mucoid, pearl-white colonies (typical of fast-growing rhizobia) were picked, restreaked on YEMA plates and stocked on YEMA slopes (Somasegaran and Hoben, 1985).

2.1.3. Salt tolerance of the isolates

To assure their tolerance to salinity, 60 nodule isolates were grown in YEM broth containing NaCl at 10 ascending concentrations ranging from 0.12 M to 1.2 M corresponding to electrical conductivities ranging from 7.02 deci Siemens per meter ($dS\,m^{-1}$) to 70.20 $dS\,m^{-1}$ as measured using a WPA-CM35 conductance-meter [Linton Cambridge WPA Scientific Instrument] and incubated for 5 days at $29 \pm 0.5\,^{\circ}C$. Salt tolerance during growth was assessed by detecting increases in the turbidity of the YEM broth following inoculation and by direct microscopy for active motility in hanging drops examined using the oil-immersion lens. Four isolates exhibiting the highest tolerance to salinity were regrown on YEMA plates having the same 10 ascending salt concentrations, incubated for 3 days at $29 \pm 0.5\,^{\circ}C$, transferred to YEMA tube slants, incubated and then kept under refrigeration for subsequent greenhouse inoculation tests, inoculant production and field inoculation tests. These four selected isolates were assigned the names RB 4/1, R/B 5/1, R/B 6/1 and R/B 7/1.

2.1.4. Test for nodulation by the selected isolates

The isolates were tested for effective nodulation on bean seedlings germinated from surface-sterilized seeds of Giza 6 under gnotobiotic tube culture conditions using agar slants containing 15 ml of sterilized half-strength Hoagland's No. 2 basal plant nutrient medium containing $(NH_4)_3PO_4$, HBO_3, $Ca(NO_3)_2$, $CuSO_4$, $Fe_4CH_4O_6$, $MgSO_4$, $MnCl_2$, MoO_3, KNO_3, $ZnSO_4$, (Sigma Chemical Co., St. Louis, MO, USA), prepared with distilled Milli-Q deionized water and solidified with 1.2% Ultrapure Agar (United States Biochemical, Cleveland, OH, USA). The tubes were incubated in a plant growth chamber programmed with a 14-h photoperiod and a $32\,^{\circ}C$ day and 10-h at $18\,^{\circ}C$ night temperature cycle. These incubation conditions for nodule establishment and development simulated the early common bean spring growing season. After 30 days of growth, root nodules were excised, surface-sterilized, macerated, and their rhizobial occupants were re-isolated on YEMA plates.

2.1.5. DNA analyses of the isolates

The taxonomic identities of the 4 isolates were evaluated at the Research Technology Support Facility at Michigan State University (http://rtsf.msu.edu/). The isolates were grown for 3 days at $29\,^{\circ}C$ in tryptone yeast extract broth in wells of microtiter plates, harvested, collected by centrifugation, resuspended in 50 μl of water and lysed by heating at $95\,^{\circ}C$ for 10 min. One μL of the lysate was used for amplification of fragments of the genomic 16 S rDNA gene using the conserved 8 F and 1492 R eubacterial primers (Edwards et al., 1989; Stackebrandt and Liesack, 1993). *Rhizobium leguminosarum* bv. trifolii strain E11 was processed identically as a positive control to validate the protocol used. The amplified products were sequenced using the 8 F primer and Applied Biosystems BigDye v3.1 Chemistry Applied Biosystems 3730xl DNA sequencing system. Sequences were aligned against those in the Ribosomal Database Project (RDP) in the Center for Microbial Ecology at Michigan State University (http://rdp.cme.msu.edu/) and evaluated by the online RDP SeqMatch sequence match tool.