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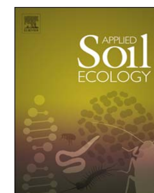

Scaling from the growth chamber to the greenhouse to the field: Demonstration of diminishing effects of mitigation of salinity in peppers inoculated with plant growth-promoting bacterium and humic acids

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ARTICLE INFO

This study is dedicated for the memory of the rhizophere researcher Dr. Michael Schmid (Big-Mike; 1968–2016) of Helmholz-Zentrum, Munich, Germany.

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ABSTRACT

The capacity of pepper plants to alleviate salt stress when inoculated with the plant growth-promoting bacteria (PGPB) *Pseudomonas stuzeri* and/or supplementation with humic acids was compared under *in vitro* conditions in a growth chamber (three independent experiments), greenhouse (four experiments) and field conditions (two experiments). Although inoculation with PGPB or humic acids significantly mitigated negative effects of salinity on germinating pepper seedlings under *in vitro* conditions, the effect was far less marked under greenhouse conditions and almost non-existent under field conditions, having no impact on yield of peppers under saline conditions. This study demonstrates that the improvement in growth with PGPB and humic acids *in vitro* may not be scalable from the laboratory to greenhouse and to field conditions. Not all PGPB improvements scale from the laboratory, greenhouse to field conditions and that the potential that laboratory results may not scale is a factor to be considered in this research field.

1. Introduction

When carrying out experiments with biotic agents with plants, a common assumption made is that experimental results or effects of biotic agents with plants will scale, that test tests under laboratory conditions will be similar to those in the greenhouse and under field condition. In agro-practice, it is not uncommon to follow a procedure of scaling-up initial experiments done in the laboratory to the field. This common ideal assumption is that experiments in the laboratory will scale linearly and indicate the best treatments to be implemented later at a large scale by the appropriate agro-industry.

While sometimes experimental conditions carried out in the laboratory are scalable and correlate well with greenhouse experiments (Bashan et al., 2009; Beattie et al., 1989; Brown et al., 1999; Kumar and Poehling, 2006; Owusu-Bennoah et al., 2002; Vleeshouwers et al., 1999; Willits and Peet, 2001), other times this is not the case. It is not uncommon at times, to find no positive correlation of an effect between the same applied experimental conditions under laboratory, greenhouse and/or field conditions (Dorrance and Inglis, 1997; Foolad et al., 2000), other times some effects are not conclusive (Kim et al., 2000) or even negative effects are observed (Antoun et al., 1998; Kesselmeier, 2001). When plant growth-promoting (rhizo)bacteria (PGPB/PGPR) are applied to increase plant performance, every major review on the topic states that PGPB/PGPR can stimulate plant growth under laboratory conditions, but when they are applied at a field-scale they fail in many cases to have the predicted impact (Bashan and de-Bashan, 2010; Bashan et al., 2014; Calvo et al., 2014; Glick, 1995; Glick et al., 2007; Martínez-Viveros et al., 2010).

Although most literature on effects of PGPB/PGPR on plant growth shows significant improvements in plant performances and yield (Bashan et al., 2014; Calvo et al., 2014; de-Bashan et al., 2012), the reality is that failures to achieve the results desired are frequent and are seldom reported, especially by businesses promoting PGPB-based inoculation technology. In an older review, it was calculated that only 70% of inoculation of various cereals with the PGPB *Azospirillum* sp. were effective (Okon and Labandera-Gonzalez, 1994), where the vast majority of the literature shows positive results.

Several of our inoculation experiments over the last two decades failed to yield the predicted positive plant response using various PGPB strains that were presumed to be potentially highly beneficial to plants when laboratory results were scaled up to the field. These results, therefore, were never published (unpublished data). As a consequence,

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our current hypothesis was that in some combinations of PGPB-plant there is no correlation between successful results obtained *in vitro* and performance of the same combination under greenhouse and field conditions. To test this hypothesis we used an experimental model employing the diazotroph PGPB *Pseudomonas stuzeri* that showed several positive responses on the growth of pepper plants under greenhouse conditions in combination with humic acids (Bacilio et al., 2016). This comparative study compared the growth of peppers under similar experimental conditions *in vitro* and in two field experiments. A general analysis was done comparing all these experiments with experimental data published before of very similar experiments done under greenhouse condition (Bacilio et al., 2016). The comparative experimental system of mitigation of salinity in peppers with humic acids and PGPB from *in vitro* studies to the field served solely as an experimental model for this comparison. Understanding the specific reasons of mitigation of salinity in peppers was not the focus of this investigation.

2. Materials and methods

2.1. Plants

Bell pepper (*Capsicum annuum* L.) cv. Jupiter (Syngenta Seeds, Boise, Idaho) and cv. Ancho San Luis (Crown Seeds, California) were used. The first cultivar is relatively tolerant to salinity and the second cultivar is relatively susceptible (Bacilio et al., 2016). Seeds were first treated with 2% Tween-20 (#P2287, Sigma, St. Luis, Mo) washed in distilled water five times, then disinfected in 3% commercial NaOCl (Clotalex, Mexico City, Mexico) for 5 min, and rinsed several times in sterile tap water. This treatment yield germination at a level of 100% within 6 days for cv. Jupiter and within 10 days for cv. Ancho San Luis.

2.2. PGPB, growth conditions and inoculant preparation

Inoculant preparation and inoculation of plants followed established guidelines (Bashan et al., 2016). The desert diazotroph PGPB *Pseudomonas stutzeri* strain TREC (GenBank accession number: JX014305; Puente and Bashan, 1994) was used for all experiments. A single colony was cultured in 250-mL Erlenmeyer flasks containing 150 mL of nutrient broth (NB, Fluka) and incubated at 32 ± 4 °C, 120 rpm for 48 h. Bacteria were harvested by centrifugation at 2683 × *g* for 10 min a 4 ± 1 °C. Bacterial cells were washed three times with saline solution (0.85%, w/v, NaCl) to eliminate all residues of nutrient broth. Finally, the bacterial suspension was diluted in the latter saline solution to 10^6 cfu mL^{-1}. This type of suspension served as inoculant for *in vitro* experiments. For experiments under field conditions, an initial suspension of 10^9 cfu mL^{-1} was the source of PGPB for formulating dry microbead alginate inoculant (100–200 μm) that was produced according to Bashan et al. (2002). This inoculant had a population of 10^8 cfu g^{-1} beads.

2.3. In vitro growth chamber screening experiments

Initially all treatments of humic acids and bacterial inoculants were screened for effectiveness in Petri dish setups. Each treatment contained four standard size Petri dishes. Each Petri dish contained two layers of towel paper and moist filter paper (Whatman #1, Sigma-Aldrich) socked with 0, 25, 50 and 75 mM NaCl (in distilled water). Seeds were disinfected as described above. Seeds were soaked for two hours in sterile distilled water. Then, 20 seeds per plate were placed on the filter paper, and incubated in a growth chamber (Conviron, Winnipeg, Canada) at 28 ± 2 °C and 80% relative humidity for the first 8 days in the dark and later under 200 μmol photon m^{-2} s^{-1}. Inoculum concentration per Petri dish (where applicable) was 10^6 CFU mL^{-1} and the concentration of commercial humic acids (active ingredients: minimum 65% humic acids and minimum 85% potassium humate; 93–98% water-soluble; Enersol SC, American Colloid, Arlington Heights, IL) was 1000 mg L^{-1}. After 21 days of incubation, the following parameters were measured: shoot length, shoot diameter, root length, number of secondary roots, length of root hairs and root hair surface. Length of root hairs and root hair surface were measured by sampling one centimeter segments of roots from the root hair zone, two centimeter above root tips (three segments per treatment). Root segments were placed in a Petri dish, stained with toluidine blue for three minutes at room temperature, and rinsed with distilled water. Finally, photomicrographs were taken under a light microscope (Olympus BX-45, Japan) connected to an image analyzer (Image ProPlus 4.5, Media Cybernetics, Silver Spring, MD) in each of these root segment chosen by random at sample size of 200 $μm^2$. The image analyzer used the software Image Pro-plus version 4.1, and photographic camera model Cool Snap Media Cybernetics. Length of root hair were measured referred to 200 $μ^2$ surface of roots.

2.4. Greenhouse experiments

Four independent greenhouse experiments having identical treatments (listed below) were done and described in detail in a previous study (Bacilio et al., 2016).

2.5. Field experiments

Two experiments under field conditions were done in the experimental field of the Guerrero Negro Experimental Station of CIBNOR located in the Vizcaíno Desert, Baja California Sur, Mexico at 27°57′32″N 114°03′22″W. This sandy desert area bordering the Pacific Ocean is characterized by having frequent fog events, annual precipitation of ∼80 mm, strong winds and high evaporation rates. The temperature during the experiments ranged between 32.8 and 6.3 °C. The soil in the area is classified as Aridosoles (Endo et al., 2000). The physicochemical characteristics of the two specific soils used in this study (saline and non-saline soils) were done by a service unit and are described in Table 1 using standard soil testing techniques authorized by the Mexican government. Soils were collected from the plough layer (upper 40 cm of the soil) from an uncultivated land at CIBNOR – Guerrero Negro Experimental Station. The soil was air-dried, grounded and screened to pass through a 2 mm sieve.

Compost used as a practical source of humic acids in these field experiments. Compost produced from cultivated cruciferous waste (cauliflower and broccoli) and dairy cow manure at a ratio of 1:2 (soil:compost, v/v, resembling the recommended level of compost application in desert soil agriculture in Baja California Sur, Mexico). The physicochemical composition and characteristics of the composts were:

Table 1
Chemical and physicochemical characteristics of the two soils used in the field experiment.

Characteristic	Non saline soil	Saline soil
Textural class	Sand	Sand
Bulk density, (g cm^{-3})	1.51–1.58	1.53–1.57
pH	8.21–9.12	8.32–9.38
EC, (dS m^{-1})	1.32–2.17	4.25–4.97
ESP (%)	6.2–11.4	23.4–31.9
$CaCO_3$ (%)	3.18–5.05	3.82–4.19
CEC, $cmol_{(+)}$ kg^{-1}	1.73–4.27	1.68–5.03
Organic matter (%)	0.19–0.38	0.14–0.45
Total nitrogen (g kg^{-1})	0.35–0.84	0.28–0.75
P-Olsen (mg kg^{-1})	30.1–44.7	24.7–41.2
Sol + Exch Ca^{2+}, (g kg^{-1})	0.63–1.23	0.73–1.88
Sol + Exch Mg^{2+} (g kg^{-1})	0.18–0.25	0.28–0.52
Sol + Exch K^+ (g kg^{-1})	0.32–0.47	0.49–0.76
Sol + Exch Na^+ (g kg^{-1})	0.12–0.38	1.61–1.93
Cl^- (g kg^{-1})	0.17–0.43	1.97–3.48

EC = Electrical conductivity; ESP = Exchangeable sodium percentage; CEC = Cation exchange capacity

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