Research Article

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Azospirillum improves lettuce growth and transplant under saline conditions

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Abstract

BACKGROUND: Recent studies have shown that as a plant-growth-promoting rhizobacteria (PGPR), *Azospirillum* inoculation could contribute to the mitigation of the negative effects caused by salt on lettuce growth. Moreover, the use of PGPR to alleviate the effects of transplant in vegetables has also been recognized. However, the scarce data available on the use of *Azospirillum* to improve lettuce growth before and after transplant under saline conditions prompted us to focus our research on this topic.

RESULTS: Early germination and seedling settlement of seeds exposed to 0 and 40 mol m^{-3} NaCl were clearly improved by *Azospirillum* inoculation. At 0 mol m^{-3} NaCl, plant establishment, leaf mass and root mass parameters before transplant were significantly higher in inoculated plants than in non-inoculated controls. At harvest, leaf fresh weight, ascorbic acid content and plant survival to transplant were also significantly higher in *Azospirillum*-inoculated plants grown at 0 mol m^{-3} NaCl. In addition to these effects, leaf dry weight, area and chlorophyll content were also increased by *Azospirillum* inoculation when plants were grown at 40 mol m^{-3} NaCl.

CONCLUSION: *Azospirillum*-inoculated lettuce seeds yield a higher number of transplanted plants with superior quality than non-inoculated controls grown at 0 or at 40 mol m^{-3} NaCl.

Keywords: *Azospirillum*; lettuce; salt; transplant

INTRODUCTION

One of the main threats to crop production at the end of the 20th century was salt stress. However, under the pressure of a growing world population; crop production is being expanded to marginal soils, where plants are frequently exposed to high salt concentrations. It has been estimated that 50% of all worldwide irrigation schemes could actually be affected by salinity.[1] Moreover, the secondary salinization of agricultural soils by irrigation is a serious land degradation problem in arid and semi-arid areas, where evaporation greatly exceeds precipitation and salts dissolved in the groundwater reach and accumulate at the soil surface through capillary movement.[2]

In particular, vegetable crops are generally more salt sensitive than grains and forages.[3] This is of concern in horticulture production in regions where water salinity may be a source of worry. Furthermore, the high impact that horticultural crops have on human nutrition is imposing a growing demand on high-quality vegetables.[4] As a consequence, the high income generated per hectare of vegetable production has justified the high cost of seeds and has stimulated the adoption of strategies to improve the cropping environment of vegetable crops.[5] However, these innovations have not prevented plants from suffering a possible salt stress associated with the support and/or the irrigation media.

Another trend in vegetable production is a gradual worldwide replacement of seed sowing in-field by nurseries, where plantlets are cultivated and grown to a manageable size. The advantages of using transplant plugs over seeds are the uniformity of plant size, ease or precision planting, and the increased vegetable production in commercial greenhouse facilities.[6] However, one of the problems a nursery has is to avoid or mitigate the negative effects that could be caused by the presence of salt in the irrigation media. Indeed, it is known that salt could have deleterious effects on the rate of germination, root elongation, seedling growth and plant establishment in lettuce.[7]

Modern agrobiotechnological strategies are being tested to enhance salt stress tolerance in plants, such as the generation of transgenic plants to introduce novel genes, or to alter expression levels of the existing genes.[5] However, a more direct approach would be to exploit the possibility of mitigating the negative effects of salt on plants by associating them with plant-growth-promoting rhizobacteria (PGPR). In this direction, it has been reported that inoculation with PGPR could increase germination, seedling emergence, growth and yield of cereal and non-cereal crops, including lettuce.[8] *Azospirillum* spp., the most studied PGPR, is able to colonize a wide range of vegetable species and to alleviate some of the negative effects of water stress and salinity on plant growth.[9] More recent results have shown the feasibility of using

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Azospirillum inoculation to mitigate the negative effects of NaCl on lettuce germination rate, root elongation, seedling growth and mature vegetative growth.[7,10] In a recent review, the need for extensive investigations tending to explore the possibility of using PGPR and other symbiotic microorganisms as strategies to facilitate sustainable agriculture on saline soils has been emphasized.[11]

A second problem associated with vegetable production in nurseries is the stress suffered by plants when transplanted to the field or to the greenhouse, which could be complicated with a concomitant salt stress taking place before and/or after transplanting. It has been suggested that previous *Azospirillum* inoculation could help plants to cope with the mechanical, environmental and physiological stresses imposed by transplant.[5] Moreover, no published study has reported the effect of *Azospirillum* inoculation on lettuce simultaneously exposed to both stresses.

The aim of this research was to study the effect of *Azospirillum* inoculation on germination, plant establishment and growth, survival rate after transplant, product quality and yield in lettuce growing at different levels of saline stress.

MATERIALS AND METHODS

Bacterial culture

Azospirillum brasilense Sp245 was cultured as described before,[7] modified as follows. Late exponential cells were centrifuged for 10 min at 8142 × *g* in a Sorvall SS43 rotor and resuspended in phosphate buffer 66 mol m^{-3} (pH 7) to obtain 0, 10^6, 10^7, 10^8, 10^9, 10^{10} and 10^{11} bacterial cell inocula per seed.

Seed inoculation

Both total liquid volume and time needed for a complete seed imbibition were calculated from time course experiments performed with *Lactuca sativa* L. cv. Elisa seeds during a 24 h period (data not shown). Seeds were surface disinfected in 1 g kg^{-1} NaOCl for 1 min, washed three times with sterile distilled water (SDW), and inoculated by immersion for 90 min in a total imbibition volume of phosphate buffer (control) or in the different bacterial inocula indicated above.

Seed germinability and seedlings sampling

A completely randomized design was used with a factorial combination of four levels of salinity and seven levels of inoculum. Each replicate was composed of 50 control seeds non-inoculated or inoculated with *Azospirillum* at 10^6, 10^7, 10^8, 10^9, 10^{10} and 10^{11} and uniformly distributed in Petri dishes lined with two sheets of filter paper (grade 37/N; Munktell Filter, Grycsbo, Sweden) previously soaked in 0, 40, 80 and 120 mol m^{-3} NaCl in SDW. Seeds were incubated in a growth chamber at 23 °C temperature and 8 h photoperiod.[12] Five replicates of each treatment were performed to determine germination percentages according to the standard germination test (SGT) for lettuce, by counting germinated seeds at 4 and 7 days after sowing (DAS).[12] At 10 DAS, the aerial part of the seedlings was removed at the base and dried in an oven at 60 °C to constant weight to determine total aerial dry weight (DW). Five replicates of each treatment were used in this experiment, each replicate composed of three samples.

Plant growth and parameter determinations

A completely randomized design was used, with a factorial combination of four levels of salinity and two levels of inoculum. Lettuce seeds were inoculated either with *A. brasilense* inocula at 10^9 cells per seed (I) or phosphate buffer (C). They were individually sown in 66-plug trays (product DI 72 E VT; Dillen, Middlefield, OH, USA), each plug containing commercial substrate (Vita Fertil, La Plata, Argentina) based on perlite, peat moss (*Musgo sphagnum*) and humus. To allow irrigation by capillarity each tray was placed in a larger tray containing the corresponding irrigating solution (0, 40, 80 and 120 mol m^{-3} NaCl). Salt concentration in the irrigating solutions were kept at set electrical conductivities by adding distilled water when required. Plugs were kept in a greenhouse under natural light (12 h approximate photoperiod). Temperature was 13–16 ± 2 °C during daytime and 11 ± 2 °C at night. Plant establishment in plugs was determined at 10, 15, 20 and 30 DAS. Three replicates of each treatment were performed, each replicate composed of 66 samples. At 35 and 45 DAS, plants were dissected in aerial and root portions and dried as described above, to obtain aerial and root DWs, respectively. Three replicates of each treatment were used in this experiment, each replicate composed of five samples.

At 45 DAS, both control and inoculated plants grown at 0 and 40 mol m^{-3} were transplanted to 5 L pots containing the same commercial substrate cited above, where growth continued for 50 more days (harvest time). A completely randomized design was used with a factorial combination of two levels of salinity and two levels of inoculum. Pots were kept in a greenhouse under natural light (12 h approximate photoperiod). Temperature was 16–19 ± 2 °C during daytime and 12 ± 2 °C at night. Irrigation and maintenance of salt concentration were performed as described above.

At 25 and 50 days after transplant (DAT), plant samples were analyzed for total leaf fresh weight (FW), area and chlorophyll content. In addition, number of leaves per plant and root DW were determined at 25 DAT, while leaf DW and ascorbic acid (AA) content were determined at harvest (50 DAT). Three replicates of each sample were used for these measurements, each replicate composed of five samples.

Chlorophyll content was determined according to the methodology described by Roura *et al.*,[13] modified as follows. At 25 and 50 DAT leaves taken at random from the middle of each plant were homogenized in a Virtis homogenizer, and 1 g replicate samples were taken from each homogenate. Each sample was then homogenized with 19 mL of a cold solution of propanone–ammonium hydroxide (18 : 1, v/v; 100 mol m^{-3}). Final homogenates were then vacuum filtered through filter paper (grade 37/N; Munktell Filter, Grycsbo, Sweden), and water was removed with anhydrous sodium sulfate. Filtrate absorbance (*A*) were measured at 660.0 and 642.5 nm in a UV 1601 PC UV–visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Total chlorophyll concentration (*C*) on each sample was calculated by applying the following formula: $C = 7.12A_{660} + 16.8A_{642.5}$. Chlorophyll content in leaves was reported as mg kg^{-1} DW.

Ascorbic acid content was determined according to Pelletier.[14] Samples of 10 g were homogenized in 6 g kg^{-1} metaphosphoric acid and filtrate aliquots titrated against 1.6-dichloroindophenol. Determinations were performed in duplicate. The content of AA was expressed as mg kg^{-1} DW.

Data were subjected to analysis of variance (ANOVA) using the SAS Version 9.0 statistical package (SAS Institute, Cary, NC, USA), and means were compared by least significant test (LSD) test ($P < 0.05$). The proportion of surviving plants at harvest for each treatment was analyzed by a chi-square homogeneity of proportion test ($P < 0.05$).

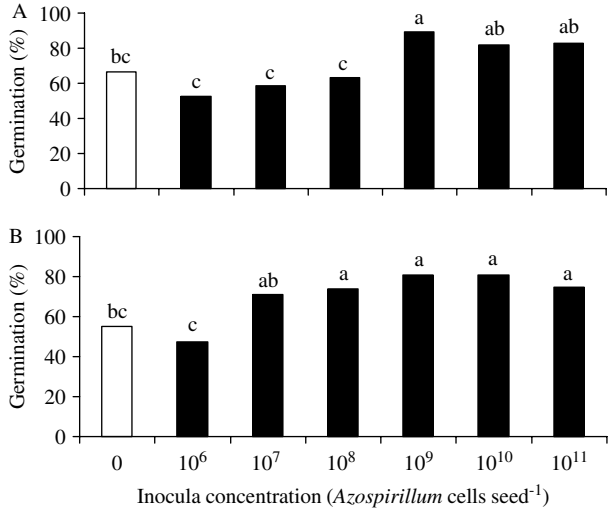

Figure 1. Early germination (4 days after sowing) in lettuce seeds previously inoculated with different *A. brasilense* Sp245 concentrations. □, control, buffer-inoculated seeds; ■, *Azospirillum*-inoculated seeds. A, germination at 0 mol m^{-3} NaCl; B, germination at 40 mol m^{-3} NaCl. Different letters on top of bars indicate significant differences in the levels of inoculum according to LSD test ($P < 0.05$).

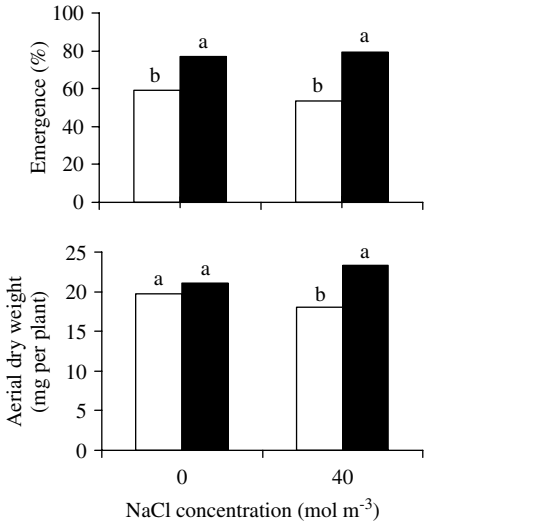

Figure 2. Emerged plantlets (10 days after sowing) obtained from lettuce seeds previously inoculated with 10^9 *A. brasilense* Sp245 cells per seed. □, control, buffer-inoculated seeds; ■, *Azospirillum*-inoculated seeds. Different letters on top of bars indicate significant differences between both levels of inocula for each salinity level according to LSD test ($P < 0.05$).

RESULTS

The effect of *Azospirillum* inoculation on lettuce growth at 0 and 40 mol m^{-3} NaCl was studied at germination, plant establishment and plant growth to harvest. This latter stage was performed after transplanting plantlets into pots. While the first stage was accomplished in a growth chamber, the last two were accomplished in the greenhouse.

Figure 1 shows the effect of different *A. brasilense* Sp245 concentrations on early lettuce seed germination exposed to 0 and 40 mol m^{-3} NaCl. A significant effect on promoting early germination was evident in both cases by inoculating with 10^9 bacteria per seed. However, no significant differences in germination were obtained at 0 mol m^{-3} NaCl when concentrations higher than 10^9 bacteria inocula per seed were used (Fig. 1).

After 10 days exposure to 0 and 40 mol m^{-3} NaCl conditions, both the number and aerial DW of plantlets emerging from *Azospirillum*-inoculated seeds were considerably higher than those obtained from non-inoculated controls (Fig. 2).

It is important to visualize how these results could be maintained or even magnified after the germination and seedling periods, i.e. to see definitive plant establishment. In this regard, Fig. 3 shows a clear *Azospirillum*'s effect on plant establishment in lettuce grown in plugs from 10 to 30 DAS.

Although root growth parameters were not evaluated in 10-day-old plants because of their small size, growth promotion on both the aerial and root parts was clearly evident in *Azospirillum*-inoculated lettuce plants at 35 and 45 DAS, that is, at the end of the second experimentation stage (Fig. 4).

A usual practice in vegetable production is to germinate seeds in plugs and then transfer plantlets to pots or to the field, where plants continue their growth. Therefore, plant tolerance to transplant and final plant survival at harvest are important factors that should not be dismissed. In order to evaluate these factors, 45-day-old plants were transplanted to pots where they continued to grow up for 50 more days, i.e. to harvest.

At 25 DAT significant results in favor of bacterial inoculation were observed in total leaf FW, leaf area, number of leaves per

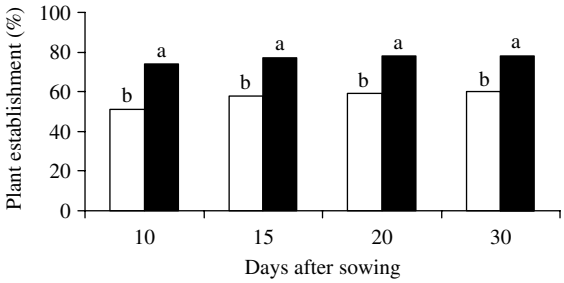

Figure 3. Lettuce plant establishment in plugs irrigated with a non-saline media (10–30 days after sowing). □, control, buffer-inoculated seeds; ■, *Azospirillum*-inoculated seeds. Different letters on top of bars indicate significant differences between treatments for each sample date according to LSD test ($P < 0.05$).

plant and chlorophyll content, and also in root DW when plants were grown at 0 mol m^{-3} NaCl (Table 1).

At harvest, the statistical differences between control and inoculated lettuce plants grown at 0 mol m^{-3} NaCl were maintained only in total leaf FW (Table 2). However, FW, DW, area and chlorophyll content in leaves of inoculated lettuce plants grown at 40 mol m^{-3} NaCl were significantly higher than in non-inoculated controls (Table 2). In addition, a clear effect of *Azospirillum* inoculation in increasing ascorbic acid content was observed in lettuce leaves harvested from plants growing either under 0 or 40 mol m^{-3} NaCl conditions (Table 2).

The data presented above (Tables 1 and 2) indicated a higher tolerance of transplanting stress for the inoculated plants than controls. This factor could account for a better plant survival at harvest. Indeed, 89% of inoculated vs. 78% of control plants growing at 0 mol m^{-3} NaCl remained alive at harvest (Table 3). This effect was more evident in lettuce grown at 40 mol m^{-3} NaCl, where 60% and 73% of plants remained alive in non-inoculated and *Azospirillum*-inoculated plants, respectively (Table 3).

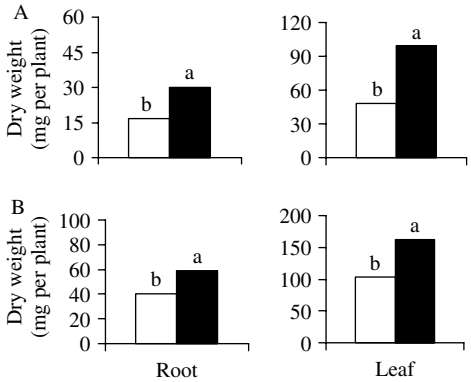

Figure 4. Lettuce mass in plants grown in pots irrigated with non-saline media. A, 35 days after sowing; B, 45 days after sowing. □, control, buffer-inoculated seeds; ■, *Azospirillum*-inoculated seeds. Different letters on top of bars indicate significant differences in different treatments according to LSD test ($P < 0.05$).

DISCUSSION

Previous results have shown an increase in seed germination at 0, 30, 50 and 80 mol m^{-3} NaCl when seeds were inoculated with 10^7 *Azospirillum* cells per seed.[7] It has been suggested that the extent of *Azospirillum*'s effect on plant growth could be dependent on the inocula concentration according to the plant species.[15] In order to evaluate early lettuce germination of seeds when exposed to 0 and 40 mol m^{-3} NaCl concentrations, six different inocula concentrations ranging from 10^6 to 10^{11} bacterial cells per seed were tested (Fig. 1). Since a significant effect on promoting early germination was evident in both cases at 10^9 bacteria per seed, we adopted this inoculum concentration as the standard for the subsequent experiments.

Considering that lettuce is a relatively salt-sensitive vegetable[7] and that 60 mol m^{-3} NaCl causes severe effects on lettuce germination rate, root elongation, seedling growth and mature vegetative growth,[7] a less severe salt stress was tried. Moreover, it has been proposed that the lowest electrical conductivity limit for a soil to be classified as saline is 4 mS cm^{-1}, which corresponds to 40 mol m^{-3} NaCl.[16] Nevertheless, none of the experiments performed at 80 and 120 mol m^{-3} NaCl provided significant differences between control and *Azospirillum*-inoculated plants (data not shown).

According to International Seed Testing Association rules, early and late germination tests of lettuce seeds should be performed at 4 and 7 DAS.[12] One of the first growth promotion effects observed on *Azospirillum*-inoculated lettuce seeds was the acceleration of the germination process.[7] In our case, this effect was evident as early as 4 DAS (Fig. 1). However, the growth-promoting effect of *Azospirillum* on early germination we observed both at 0 and 40 mol m^{-3} NaCl does not necessary imply a similar effect on seedling emergence. In this regard, in several vegetables tolerance to salt at one growth stage is not correlated to tolerance at another one.[3] Indeed, Hela *et al.*[17] showed that two different lettuce varieties (Verte and Romaine) could invert their relative tolerance to salt when both plants grow beyond germination and early seedling establishment, i.e. up to 26 DAS. In spite of that, 10 DAS at 0 and 40 mol m^{-3} NaCl both the number and aerial DW of plantlets emerging from *Azospirillum*-inoculated seeds were considerably higher than those obtained from non-inoculated controls (Fig. 2). Root DW was too low at this stage to be accurately

Table 3. Plant survival at harvest (50 days after transplant)

Treatment			
0 mol m^{-3} NaCl		40 mol m^{-3} NaCl	
Control	Inoculated	Control	Inoculated
78%	89%	60%	73%

The proportion of surviving plants at harvest for each treatment is different ($P < 0.05$).

Table 1. Growth parameters in 70-day-old lettuce plants transplanted at 45 days after sowing and irrigated with a non-saline media

	Growth parameters				
Inocula	Leaf FW (g per plant)	Leaf area (cm^2 per plant)	Leaf amount (leaves per plant)	Chlorophyll (mg kg^{-1} DW)	Root DW (g per plant)
Control	44.6 ± 2.2b	914.0 ± 56.8b	17.3 ± 0.6b	37.7 ± 0.4b	2.1 ± 0.3b
Azospirillum	57.0 ± 2.8a	1124.1 ± 49.7a	20.8 ± 0.5a	54.4 ± 1.3a	4.3 ± 0.9a

± mean SEM ($P < 0.05$). Different letters mean significant differences ($P < 0.05$).

Table 2. Harvest parameters in 95-day-old lettuce plants transplanted at 45 days after sowing and irrigated either with 0 or 40 mol m^{-3} NaCl solutions

		Growth parameters at harvest				
NaCl (mol m^{-3})	Inocula	Leaf FW (g per plant)	Leaf DW (g per plant)	Leaf area (m^2 per plant)	Chlorophyll (mg kg^{-1})	AA (mg kg^{-1})
0	Control	207.2 ± 6.1b	9.85 ± 0.85ab	0.36 ± 0.01ab	82.4 ± 3.9ab	15.1 ± 1.2c
	Azospirillum	240.9 ± 4.1a	11.77 ± 1.12a	0.42 ± 0.04a	86.6 ± 1.1a	26.6 ± 3.3ab
40	Control	101.6 ± 7.0d	3.58 ± 0.59c	0.16 ± 0.02c	70.3 ± 2.9b	20.6 ± 1.9bc
	Azospirillum	126.8 ± 5.7c	7.22 ± 0.32b	0.26 ± 0.02b	86.9 ± 3.6a	30.7 ± 1.4a

± mean SEM ($P < 0.05$). Different letters mean significant differences ($P < 0.05$).

determined (data not shown). Moreover, at 30 DAS the percent of established plants in plugs at 0 mol m^{-3} NaCl was statistically higher in *Azospirillum*-inoculated plants than in controls (Fig. 3). These results are in agreement with data obtained before[7] and imply that *Azospirillum* inoculation has growth-promoting effects on lettuce that last well over the germination and early seedling stages.

The remarkable effect *Azospirillum* inoculation exerted on root growth promotion has been early reported as one of the reasons why these plants could tolerate water stress more successfully than non-inoculated controls.[18] Such a root growth effect was clearly evident in *Azospirillum*-inoculated lettuce plants at 35 and 45 DAS, i.e. at the end of the second experimentation stage (Fig. 4). Moreover, a well-developed root system could be an important factor in mitigating transplanting stress. In fact, leaf weight, area and chlorophyll content in *Azospirillum*-inoculated lettuce were significantly higher than controls in plants grown at 0 mol m^{-3} NaCl during 25 DAT (Table 1).

At harvest (140 DAS), total FW and ascorbic acid content in leaves of inoculated lettuce plants grown at 0 mol m^{-3} NaCl were significantly higher than in non-inoculated controls (Table 2). In terms of vegetable production, this could imply obtaining individual plants having both an enhanced edible part and a higher nutritional value. These effects associated with *Azospirillum* inoculation, plus clear increases in leaf DW, area, and chlorophyll content, were also observed in harvested plants grown at 40 mol m^{-3} NaCl (Table 2). At this NaCl concentration, harvested plants may have suffered not only saline but also transplanting stresses.

Regarding transplanting stress alone, the data presented above (Tables 1 and 2) indicate that inoculated plants could perform better than controls at 0 mol m^{-3} NaCl. This factor could account for a better plant survival at harvest. Indeed, 89% of inoculated vs. 78% of control plants growing at 0 mol m^{-3} NaCl remained alive at harvest (Table 3). This effect was more evident in lettuce grown at 40 mol m^{-3} NaCl, where 60% and 73% of plants remained alive in non-inoculated and *Azospirillum*-inoculated plants, respectively (Table 3).

Finally, a rough approximation of the effect of *Azospirillum* inoculation on lettuce yield produced under the conditions reported here could be calculated from leaf FW data at harvest (Table 2), the potential loss due to plant dying after transplant (Table 3), and an estimated plant density of 16 000 plants ha^{-1}. Within these considerations, at 0 mol m^{-3} NaCl the numbers obtained from control and inoculated plants were 2.6 and 3.4 ton ha^{-1} respectively, representing a 33% gain in favor of inoculation. This effect was more pronounced at 40 mol m^{-3} NaCl, where 1.0 vs. 1.5 ton ha^{-1} were obtained from control and inoculated plants respectively, thus representing a theoretical 52% yield gain in *Azospirillum*-inoculated plants over non-inoculated controls.

Even though we do not provide data that could explain the observed *Azospirillum* effects, one of the most accepted ideas follows this line: root growth stimulation → increased water and mineral absorption → better water status → healthier plants.[9,19] In turn, root growth stimulation could be associated with the bacterial ability to produce different plant growth substances such as auxins, cytokinins and gibberellins[20] and to induce the transformation of inactive to active phytohormones.[21] However, other plausible explanations regarding not only root growth promotion but also plant protection from stresses could be associated with *Azospirillum*'s versatility under different circumstances. Indeed, mineral solubilization and enhanced mineral uptake,[9,19] nitrite production,[19] nitrate reduction,[22] exopolysaccharide production,[23] siderophore synthesis,[24] modification of plant enzymes involved in Krebs and glycolysis pathways[25] and increase of organic solutes that contribute to plant osmoregulation[26] are well-known examples of the ample set of abilities displayed by *Azospirillum*. More insights into advances in the understanding of physiological properties of *A. brasilense* are provided in a recent review.[27] Finally, in the absence of a definitive agreement on exactly how *Azospirillum* can exert its beneficial effects on plants, an interesting unifying 'multiple mechanism theory', where not a single bacterial ability but rather a combination of them participates in promoting plant growth in each inoculation case, has been recently proposed.[19]

In short, a growth promotion effect of *Azospirillum* inoculation on lettuce exposed to 0 mol m^{-3} NaCl was observed at early germination (Fig. 1), seedling settlement (Fig. 2) and definitive plant establishment stages (Figs 3 and 4). Moreover, *Azospirillum* inoculation also had a beneficial effect in protecting plants from transplanting stress (Tables 1 and 2). All these effects could possibly account for enhanced plant survival and quality (Table 3) and thus result in better yield at harvest.

CONCLUSION

In this study, *Azospirillum*-inoculated lettuce seeds yielded a higher number and mass of transplanted plants with superior quality than non-inoculated controls grown at 0 and at 40 mol m^{-3} NaCl. The results presented here could justify more studies to explore the possibility to install nurseries and/or grow vegetables in world regions where the salt presence could be troublesome. However, further research is required in order to gain a more complete understanding of the bacterial mode of action.

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REFERENCES

1 Hu Y and Schmidhalter U, Drought and salinity: a comparison of their effects on mineral nutrition of plants. *J Plant Nutr Soil Sci* **168**:541–549 (2005).
2 Kohler J, Caravaca F and Roldán A, An AM fungus and a PGPR intensify the adverse effects of salinity on the stability of rhizosphere soil aggregates of *Lactuca sativa*. *Soil Biol Biochem* **42**:429–434 (2010).
3 Shannon MC, Adaptation of plants to salinity. *Adv Agron* **60**:75–120 (1997).
4 Picha D, Horticultural crop quality characteristics important in international trade. *Acta Hortic* **12**:423–426 (2006).
5 Barassi CA, Sueldo RJ, Creus CM, Carrozzi LE, Casanovas EM and Pereyra MA, *Azospirillum* spp., a dynamic soil bacterium favourable to vegetable crop production. *Dynam Soil Dynam Plant* **1**:68–82 (2007).
6 Kloepper JW, Reddy MS, Kenney DS, Vavrina C, Kokalis-Burelle N and Martinez-Ochoa N, Applications for rhizobacteria in transplant production and yield enhancement. *Acta Hortic* **631**:219–229 (2004).
7 Barassi CA, Ayrault G, Creus CM, Sueldo RJ and Sobrero MT, Seed inoculation with *Azospirillum* mitigates NaCl effects on lettuce. *Sci Hortic Amsterdam* **109**:8–14 (2006).

8 Scuderi D, Giuffrida F, Cassaniti C, Caturano E and Leonardi C, Contribution of beneficial rhizosphere micro-organisms to growth and nitrogen uptake of lettuce plants under field conditions. *J Food Agric Environ* **9**:689–692 (2011).
9 Bashan Y, Holguin G and de-Bashan LE, *Azospirillum*–plant relationships: physiological, molecular, agricultural, and environmental advances (1997–2003). *Can J Microbiol* **50**:521–577 (2004).
10 Yildirim E, Turan M, Ekinci M, Dursun A and Cakmakci R, Plant growth promoting rhizobacteria ameliorate deleterious effect of salt stress on lettuce. *Sci Res Essays* **6**:4389–4396 (2011).
11 Singh JS, Pandey VC and Singh DP, Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. *Agric Ecosyst Environ* **140**:339–353 (2011).
12 International Seed Testing Association, The germination test, in *Proceedings of the International Seed Testing Association*. International Seed Testing Association Press, Zurich, pp. 155–199 (1999).
13 Roura SI, Moreira MR, Ponce AG and del Valle C, Dip treatments for fresh romaine lettuce. *Ital J Food Sci* **3**:405–415 (2003).
14 Pelletier O, Vitamin C: l-Ascorbic and dehydro-l-ascorbic acids, in *Methods of Vitamin Assay*, ed. by Augustin J, Klein BP, Becker D and Venugopal BP. Wiley, New York, pp. 1–590 (1985).
15 Okon Y and Labandera-González CA, Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. *Soil Biol Biochem* **26**:1591–1601 (1994).
16 United States Department of Agriculture, Diagnosis and improvement of saline and alkali soils, in *Agriculture Handbook*, Vol. 60. USDA, Washington, DC (1954).
17 Mahmoudi H, Kaddour R, Huang J, Nasri N, Olfa B, Hannoufa SMA, *et al*, Varied tolerance to NaCl salinity is related to biochemical changes in two contrasting lettuce genotypes. *Acta Physiol Plant* **33**:1613–1622 (2011).
18 Okon Y and Kapulnik Y, Development and function of *Azospirillum*-inoculated roots. *Plant Soil* **90**:3–16 (1986).
19 Bashan Y and de-Bashan LE, How the plant growth-promoting bacterium *Azospirillum* promotes plant growth: a critical assessment. *Adv Agron* **108**:77–136 (2010).
20 Cohen AC, Travaglia CN, Bottini R and Piccoli PN, Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. *Botany* **87**:455–462 (2009).
21 Cassán F, Bottini R, Schneider G and Piccoli P, *Azospirillum brasilense* and *Azospirillum lipoferum* hydrolyze conjugates of GA20 and metabolize the resultant aglycones to GA1 in seedlings of rice dwarf mutants. *Plant Physiol* **125**:2053–2058 (2001).
22 Steenhoudt O and van der Leyden J, *Azospirillum* a free-living nitrogen fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. *FEMS Microbiol Rev* **24**:487–506 (2000).
23 Upadhyay SK, Singh JS and Singh DP, Exopolysaccharide-producing plant growth-promoting rhizobacteria under salinity condition. *Pedosphere* **21**:214–222 (2011).
24 Tortora ML, Diaz-Ricci JC and Pedraza RO, *Azospirillum brasilense* siderophores with antifungal activity against *Colletotrichum acutatum*. *Arch Microbiol* **193**:275–286 (2011).
25 Fallik E, Sarig S and Okon Y, Morphology and physiology of plant roots associated with *Azospirillum*, in *Azospirillum/Plant Association*, ed. by Okon Y. CRC Press, Boca Raton, FL, pp. 77–86 (1994).
26 Hamdia MAE, Shaddad MAK and Doaa MM, Mechanisms of salt tolerance and interactive effects of *Azospirillum brasilense* inoculation on maize cultivars grown under salt stress conditions. *Plant Growth Regul* **44**:165–174 (2004).
27 Fibach-Paldi S, Burdman S and Okon Y, Key physiological properties contributing to rhizosphere adaptation and plant growth promotion abilities of *Azospirillum brasilense*. *FEMS Microbiol Let* **326**:99–108 (2012).

