

Seed inoculation with *Azospirillum* mitigates NaCl effects on lettuce

C.A. Barassi^{a,*}, G. Ayrault^b, C.M. Creus^a, R.J. Sueldo^a, M.T. Sobrero^b

^a *Unidad Integrada Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata-EEA-INTA Balcarce, C.C. 276-INTA, Balcarce 7620, Argentina*

^b *Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero, Argentina*

Received 24 June 2005; received in revised form 24 February 2006; accepted 27 February 2006

Abstract

Data on the growth-promoting effects of *Azospirillum* on lettuce exposed to either normal or saline conditions, is scarce. *Lactuca sativa* L., cv Mantecosa seeds were colonized with *A. brasilense* Sp245 cells during imbibition. Germination percentages were determined after 7 d treatments with 0, 30, 50 or 80 mol m⁻³ NaCl. In another experiment, seeds germinated in Hoagland were irrigated for 30 d with 0, 30, 50 or 80 mol m⁻³ NaCl supplemented media. Vegetative growth proceeded in a growth chamber with a 13–11 h day–night cycle. Buffer-imbibed seeds were considered non-inoculated controls. Plant samples were taken at 0, 14, 20, and 30 d after the onset of NaCl treatments and dissected in aerial and root portions. The weights of both tissues were measured. *Azospirillum*-inoculated seeds had significantly higher germination percentages than controls in all treatments. Inoculated dried seeds stored up to 30 d maintained such characteristic in most of the treatments, particularly at 80 mol m⁻³ NaCl. Plants grown from inoculated seeds and irrigated with saline media displayed higher total fresh and dry weights and biomass partition to the aerial portion, than non-inoculated controls. *Azospirillum*-inoculated lettuce seeds had better germination and vegetative growth than non-inoculated controls after being exposed to NaCl.

© 2006 Elsevier B.V. All rights reserved.

Keywords: *Azospirillum*; *Lactuca sativa*; NaCl

1. Introduction

Field salinization is a growing problem worldwide. It was estimated that 10% of the world's cropland and as much as 27% of the irrigated land may be already affected by salinity (Shannon, 1997). A more recent review quotes that one-third of the world's arable land resources are affected by salinity (Qadir et al., 2000). Not surprisingly, the gradual increase in salt content in irrigated soils has been considered as one of the main threats against crop production (Kotb et al., 2000). Vegetable crops are generally more salt sensitive than grains and forages (Shannon, 1997). To make things worse, vegetable crops are grown widely in regions where irrigation water salinity may have long-term negative effects on soil–water–plant relationships (Graifenberg et al., 1993). Even when there are wide differences in the tolerance to salt among cultivars (Shannon et al., 1983; Coons et al., 1990), *Lactuca sativa* is considered to be a relatively salt

sensitive vegetable (Martínez et al., 1996), more than broccoli, cucumber, spinach, cabbage and pepper, but less than carrots, onions and radish (Xu et al., 2000). High (60 mol m⁻³) NaCl in nutrient solution strongly affected the germination rate and root elongation, seedling and mature vegetative growth of both spinach and lettuce, but especially in lettuce (Kaya et al., 2002). At 80 mol m⁻³ NaCl, lettuce germinability is reduced in a 50% (Odegaro and Smith, 1969). Even when salt seemed to affect lettuce growth mainly through osmotic effects (Shannon, 1997), recent results have shown that plant performance under different NaCl treatments was affected both by ionic and osmotic effects (Tarakcioglu and Inal, 2002). Moreover, salt–nutrient interactions could also account for the NaCl negative effects on plant growth and yield quality (Martínez et al., 1996; Pardossi et al., 1999), or for improving plant performance under saline conditions (Kaya et al., 2002).

On the other hand, the use of plant growth-promoting bacteria (PGPB) and mycorrhizal fungi to promote plant growth in saline soils is a developing technology (Hamdi, 1999; Bacilio et al., 2004). In general, inoculation with PGPB can enhance germination, seedling emergence and modify growth

* Corresponding author. Tel.: +54 2266 43 9100x292; fax: +54 223 474 9009.
E-mail address: cbarassi@balcarce.inta.gov.ar (C.A. Barassi).

and yield of various cereal and non-cereal crops (Zahir et al., 2004). Regarding *Azospirillum*, the most researched associative bacterium (Bashan and Holguin, 1997), stress conditions appear to emphasize its growth-promoting effects on plants (Barassi et al., 2000). As pointed in the 80s, *Azospirillum* could be efficiently used in crops that are not commonly irrigated in semiarid zones, improving root growth and thus, plant water status (Okon, 1985). *Azospirillum brasilense* Sp245 was able to mitigate water stress effects in wheat seedlings (Alvarez et al., 1996; Creus et al., 1998) and maize (Casanovas et al., 2002). These favourable effects were also evident at the field (Casanovas et al., 2003; Creus et al., 2004). Moreover, semiarid agriculture is frequently associated with increased soil salinization, situation that could be partially overcome by *Azospirillum* inoculation (Bashan et al., 2004). Indeed, it has been shown that the damaging effects of NaCl on wheat seedlings could be reduced by inoculation with *A. brasilense* (Creus et al., 1997) or a genetically modified *A. lipoferum* (Bacilio et al., 2004). The favourable effects of *Azospirillum* inoculation were also observed in chickpea irrigated with saline water (Hamaoui et al., 2001).

Taking into account such considerations and the well known fact that plants are often more sensitive to salinity during germination and seedling growth stages (Al-Karaki, 2001), we explored the possibility of using *A. brasilense* Sp245 to mitigate NaCl effects on a sensitive plant species such as *L. sativa*. In short, the objectives of the present investigation were to determine whether: (a) *A. brasilense* Sp245 could be inoculated in pregerminating lettuce seeds; (b) *Azospirillum* inoculation could improve the germination percentage in lettuce seeds exposed up to 80 mol m⁻³ NaCl germinating solutions; (c) the effects of *Azospirillum* on germination could be maintained after the drying and 30 d storage of inoculated seeds; (d) *A. brasilense*-inoculated lettuce plants could grow better under saline conditions than non-inoculated plants.

2. Material and methods

2.1. Bacterial culture

A. brasilense Sp245 was kept in agar–Congo red medium (Rodríguez Cáceres, 1982), transferred to Okon, Albrecht and Burris (OAB, 1977) liquid medium containing 0.1% NH₄Cl, and incubated at 32 °C with orbital agitation (100 rpm). To build the bacterial growth curve, both optical density at 600 nm, and most probable numbers (MPN) in semisolid NFb medium (Döbereiner and Day, 1976; Postgate, 1969), were determined in 1 mL samples taken every hour and for a total 24 h period (data not shown). A 10⁷ cells seed⁻¹ inoculum was obtained by centrifuging late exponential cells (10 min at 8142 × g) in a SS34 Sorvall rotor, followed by resuspension in 66 mol m⁻³ phosphate buffer (pH 7).

2.2. Seed inoculation and storage

The water percentage incorporated into seeds versus the time and the total imbibition volumes were calculated from

imbibition curves determined for *Lactuca sativa* L. cv Mantecosa seeds during a 24 h period (data not shown).

Seeds were surface-disinfected in 1% NaOCl for 5 min, washed thrice with sterile distilled water (SDW), and inoculated by immersion for 3 h in a total imbibition volume of phosphate buffer (control) or bacterial inoculum containing 10⁷ bacterial cells seed⁻¹. Following drying to 50% humidity under a 30 °C air flow, seeds were arranged in one-layer beds and left in the laminar flow cabinet to complete the desiccation process. Dried seeds were stored up to 30 d in paper bags at room temperature (20 ± 2 °C) in a dark, dry place.

2.3. Colonization assessment

After 0, 15, and 30 d of storage, seeds were homogenized in a sterile mortar with phosphate buffer. Serial dilutions in phosphate buffer were obtained from each sample. Three replicates from each dilution were cultured in semisolid NFb medium containing 0.1% NH₄Cl and bacterial MPN per gram of fresh weight (FW) was estimated according to Postgate (1969). Bacterial growth was then transferred to agar–Congo red medium to detect stained colonies similar to those obtained with pure *A. brasilense* cells (Rodríguez Cáceres, 1982).

A similar protocol was used to determine bacterial MPN in seedlings 7 d after sowing (DAS).

2.4. Seed germinability

Each replicate was composed of 50 *Azospirillum*-inoculated or non-inoculated seeds uniformly distributed on a single sheet of filter paper (Munktell Filter, Grycsbo, Sweden; grade 37/N), adequately wetted either with 0, 30, 50, or 80 mol m⁻³ NaCl in SDW. Every sheet was covered with another equally wetted paper, wrapped in parafilm and aluminum foil, and incubated in a germination incubator at 20 °C. Seed germination was determined according to the standard germination test (SGT) for lettuce, by counting germinated seeds at four and seven DAS imbibed seeds (International Seed Testing Association, 1999). Results corresponding to final counts (seven DAS) were reported as germination percentages. Similar experiments were performed with dried seeds stored for 0, 15, and 30 d.

Bacterial presence in germinated seeds was determined as described above in 50 seeds from each treatment, sampled at seven DAS.

2.5. Plant growth under saline conditions

After 2 d inoculation either with *A. brasilense* or phosphate buffer (control), three lettuce seeds plug⁻¹ were sown in 72-plug trays (Dillen, Middlefield, Ohio, USA; DI 72 E VT product), each plug containing a 1:1 v:v mixture of sand and vermiculite. To allow irrigation by capilarity, each tray was put into a larger tray containing the corresponding irrigating solution. After seven DAS irrigation with plain Hoagland's, plant number was thinned to 1 per plug and nutrient solution replaced by either 0, 30, 50, or 80 mol m⁻³ NaCl in Hoagland's. The salt concentration was controlled by electrical conductivity

and kept constant by adding distilled water to the irrigation solution when necessary. Plant growth proceeded in a growth chamber up to a total of 37 d under fluorescent and incandescent lights, with a 13–11 h day–night cycle, and at a photon flux density of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the plug's periphery. Plant samples were taken at 0, 14, 20, and 30 d after the onset of NaCl treatments. Plants were weighed for fresh weight (FW), dissected in aerial (A) and root (R) portions. Dry weight (DW) was determined in both tissues after being dried in an oven at 60°C up to constant weight. Both FW and DW data were used to determine weight vs time curves. The area under each weight–gain curve (AWGC) was calculated in a total period of 30 d, as follows:

$$\text{AWGC} = \sum_{i=1}^n [(Y_{i+n_i} + Y_i) - 2][X_{i+1} - X_i],$$

where Y_i = weight at the i th observation; X_i = time (d) at the i th observation; and n = total number of observations. The DW figures obtained by this method were also used to calculate biomass partition (BP), as $\text{BP} = \text{AerialDW}/(\text{RootDW})^{-1}$.

2.6. Experimental design and statistical analyses

The germination experiment was a factorial combination of four salt concentrations, three storage times, and two inoculation levels arranged in a completely randomized design, with ten replicates. Angular transformation was applied to all percentage data to homogenise variances. The experiment concerning plant growth was a factorial combination of four salt concentrations, four evaluation times, and two inoculation levels arranged in a completely randomized design, with three replicates.

Data were subjected to ANOVA using the SAS statistical software package (SAS Institute, 2000) and means were compared by LSD test ($P < 0.05$).

3. Results

According to the imbibition curves, lettuce incorporated water to a maximum of 0.8 mL g^{-1} FW of seeds after 3 h soaking (data not shown). During this period, seeds imbibed with the above volume of phosphate buffer containing 10^7 bacterial cells seed^{-1} , successfully incorporated 10^6 viable bacteria seed^{-1} . Moreover, *Azospirillum* MPN remained unchanged at 30 mol m^{-3} NaCl, diminishing in one order of magnitude at 50 or 80 mol m^{-3} NaCl (Fig. 1a). Less than 100 MPN seed^{-1} were found in control seeds (Fig. 1a).

As indicated by the SGT and at 0 mol m^{-3} NaCl, *Azospirillum*-inoculated seeds had a 10% increase in the germination percentage over its non-inoculated control (Fig. 1b). On the other hand, seeds exposed to 30, 50 and 80 mol m^{-3} NaCl experienced a 4.1%, 11.5% and a 87.5% drop in germination percentage, respectively (Fig. 1b). In contrast, compared to the non-inoculated control at 0 mol m^{-3} NaCl, the germination percentage had a 11.2% increase at 30 mol m^{-3} NaCl, no drop at 50 mol m^{-3} NaCl, and only a 15.6% fall in

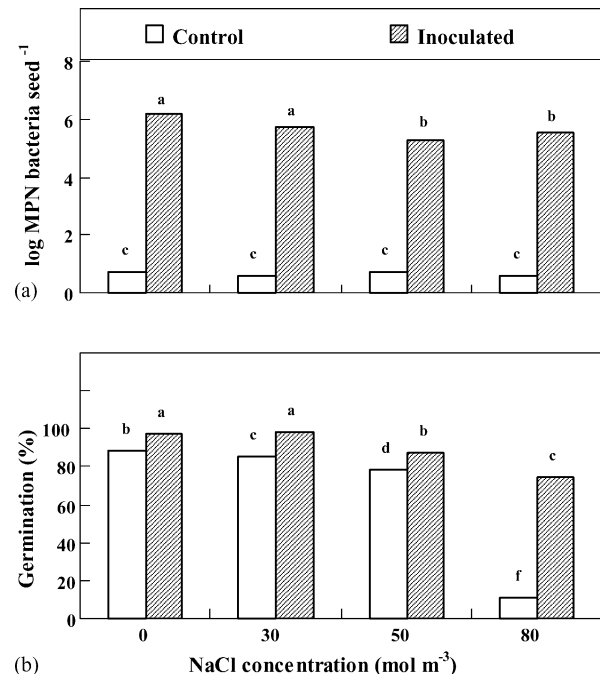


Fig. 1. Most probable number of microaerophilic bacteria in lettuce seeds inoculated with *A. brasilense* Sp245 and germinated at different NaCl concentrations during 7 d. (a) Germination percentage in non-inoculated and *Azospirillum*-inoculated lettuce seeds after 7 d exposure to different NaCl concentrations, (b) different letters on top of bars indicate significant differences according to LSD test ($P < 0.05$).

Azospirillum-inoculated seeds (Fig. 1b). In other terms, inoculating lettuce seeds with *Azospirillum* during imbibition could revert in a 71.9%, the negative effects caused by 80 mol m^{-3} NaCl on the germination percentage.

After inoculation, seeds could be dried, stored up to 30 d, and still be able to withstand high salinity levels during germination. In effect, Fig. 2 shows that except those seeds

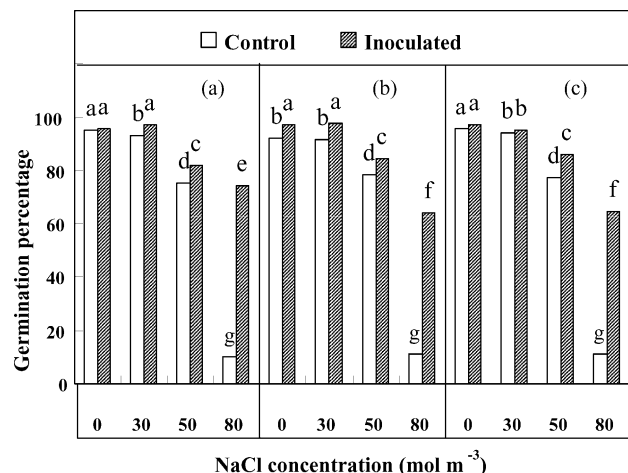


Fig. 2. Storage time effect on germination at different NaCl concentrations, in lettuce seeds inoculated with *A. brasilense* Sp245 during imbibition and dried immediately thereafter. (a) 0 d Storage; (b) 15 d storage; (c) 30 d storage. Different letters on top of bars indicate significant differences according to LSD test ($P < 0.05$).

stored during 30 d and then exposed to 30 mol m^{-3} NaCl, *Azospirillum*-inoculated seeds had significantly higher germination percentages than non-inoculated controls (Fig. 2). Such results reveal a 67.7%, 57.5%, and 56.2% reversion of the adverse effects caused by 80 mol m^{-3} NaCl on the germination percentage after 0, 15 and 30 d storage, respectively (Fig. 2). In general, germination percentages remained over 60% in inoculated seeds at all the NaCl concentrations and storage times tested (Fig. 2).

Independently of the effect *Azospirillum* inoculation had in reverting the adverse effects that NaCl had shown on the germination percentages, it was interesting to observe whether such property could be extended beyond the germination period. In this regard, 7 d-old lettuce plants were grown at 0, 30, 50, and 80 mol m^{-3} NaCl and sampled for FW and DWs at 0, 14, 20, and 30 d after the onset of NaCl treatments.

The analysis of variance indicated a triple significant interaction among salinity, time, and inoculation factors

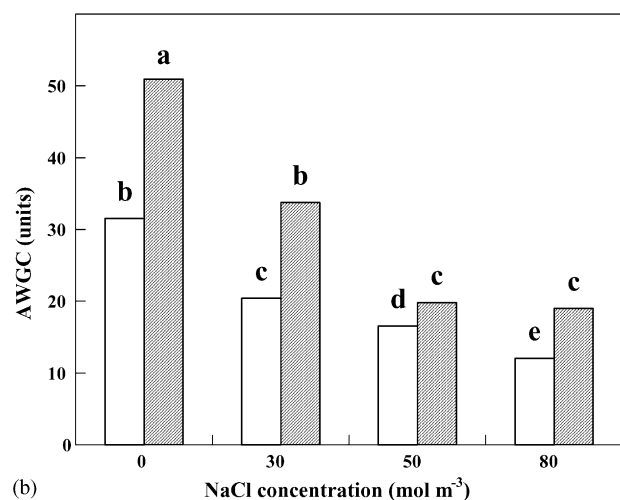
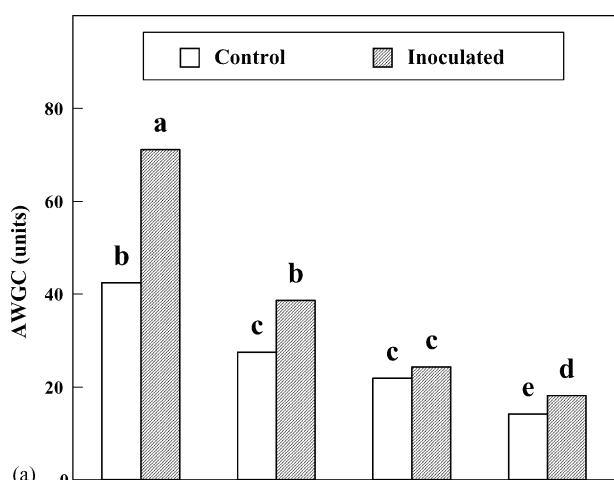


Fig. 3. Fresh and dry weight progression of *Azospirillum*-inoculated and non-inoculated lettuce plants grown for 30 d at 0, 30, 50, and 80 mol m^{-3} NaCl. Results are expressed as areas under the weight gain curves (AWGC). (a) Fresh weight; (b) dry weight. Different letters on top of bars indicate significant differences according to LSD test ($P < 0.05$).

(data not shown). When the statistical analysis was opened by time factor, a non-significant interaction between salinity and inoculation was found (data not shown). A useful means of obtaining an overall view of a process where samples are taken consecutively, is to express results as areas under the progress curves (Shaner and Finney, 1977). Taking into account such consideration, in order to unify the time factor and to determine the effect of bacterial inoculation on plant growth at different NaCl concentrations, AWGC were obtained and analysed by ANOVA. In general, NaCl significantly reduced weight gain at all the concentrations used in the present work. However, there were no significant differences in FW gain between plants exposed to 30 and 50 mol m^{-3} NaCl (Fig. 3a). In contrast, DW gain was progressively reduced with the increase in NaCl concentration (Fig. 3b). Regarding the effects of *Azospirillum*, inoculated plants (except at 50 mol m^{-3} NaCl) had significantly higher FW gain than their respective non-inoculated controls after 30 d growth (Fig. 3a). Moreover, *Azospirillum*-inoculated plants grown at 30 mol m^{-3} NaCl had the same FW gain as non-inoculated controls at 0 mol m^{-3} NaCl (Fig. 3a). Such growth-promoting effect was more evident on the plant DW. In effect, *Azospirillum*-inoculated plants grown at 50 and 80 mol m^{-3} NaCl had the same DW gain as non-inoculated controls at 30 mol m^{-3} NaCl (Fig. 3b).

Since the marketable part of the lettuce plant is the aerial portion, it was interesting to study how NaCl affected BP and the effect of *Azospirillum* on such response. Under non-saline conditions, BP to the aerial portion experienced a 56% increase in *Azospirillum*-inoculated plants when compared to non-inoculated controls (Fig. 4). A significant BP drop in plants exposed to 80 mol m^{-3} NaCl, was observed after 30 d treatment (Fig. 4). In contrast, *Azospirillum*-inoculated plants maintained roughly the same BP as non-inoculated plants at 0 mol m^{-3} NaCl (Fig. 4).

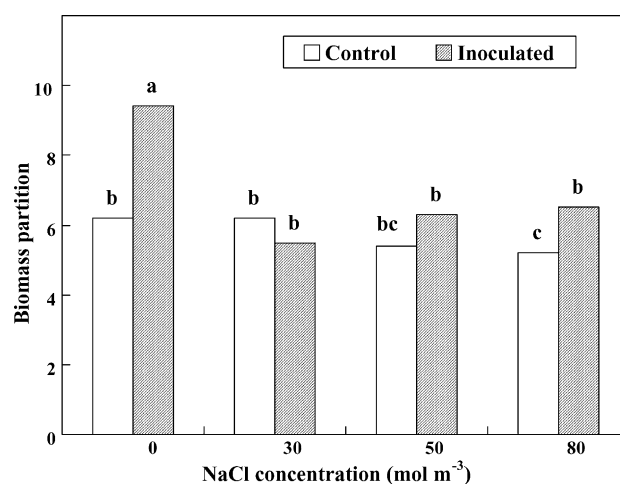


Fig. 4. Biomass partition (BP) to the aerial portion, in *Azospirillum*-inoculated and non-inoculated lettuce plants grown for 30 d at 0, 30, 50, and 80 mol m^{-3} NaCl. Biomass partition was calculated from DW data, according to the formula: $\text{BP} = \text{AerialDW}(\text{RootDW})^{-1}$. Different letters on top of bars indicate significant differences according to LSD test ($P < 0.05$).

4. Discussion

By imbibing seeds with *Azospirillum* cells suspended in a predetermined amount of phosphate buffer (Creus et al., 1996; Casanovas et al., 2000), we were able to introduce 10^6 bacterial cells per *L. sativa* seed. Moreover, *Azospirillum* MPN remained unchanged in roots at 30 mol m^{-3} NaCl, diminishing in one order of magnitude at 50 or 80 mol m^{-3} NaCl (Fig. 1a). Such results indicate an effective colonization even when germination and seedling growth took place in high NaCl concentrations. This would indicate a relatively high tolerance of *A. brasilense* Sp245 to salt when compared to other microaerophilic bacteria (Hartmann, 1988; Bashan and Holguin, 1997). Therefore, it was interesting to observe whether the well known plant growth-promoting effect of *Azospirillum* could also be evident in lettuce seeds germinating in both non-saline and saline conditions.

At 0 mol m^{-3} NaCl concentration, germination percentage was significantly increased in *Azospirillum*-inoculated seeds (Fig. 1b). On the other hand, germination percentage decreased significantly with each increase in NaCl concentration (Fig. 1b). The most negative effect of salt was observed at 80 mol m^{-3} NaCl, where seed germination dropped from 88.6% to 11.1% (Fig. 1b), that is, to well below the LD_{50} (Rehman et al., 1999). In contrast, at the same NaCl concentration the germination percentage had fallen from 97.6% to 74.8% in *Azospirillum*-inoculated seeds. In other terms, if we compare data obtained with non-inoculated and inoculated seeds exposed to the highest salt concentration to that of non-inoculated controls at 0 mol m^{-3} NaCl, we could conclude that *Azospirillum* inoculation could revert in a 71.9%, the adverse effects caused by 80 mol m^{-3} NaCl on lettuce germination. At lower NaCl concentrations the effect of *Azospirillum* is less marked but still evident when comparing inoculated seeds to their respective non-inoculated controls. Moreover, the germination percentage in *Azospirillum*-inoculated seeds exposed to 30 and 50 mol m^{-3} NaCl is, respectively, higher and equal to non-inoculated seeds germinating at 0 mol m^{-3} NaCl (Fig. 1b).

Additionally, inoculated seeds could be dried, stored up to 30 d, and still be able to withstand high salinity levels during germination. In general, germination percentages remained over 60% in inoculated seeds at all the NaCl concentrations and storage times tested (Fig. 2). In particular, *Azospirillum* inoculation reverted in a 67.7%, 57.5%, and 56.2% the adverse effects caused by 80 mol m^{-3} NaCl on the germination percentage after 0, 15 and 30 d storage, respectively (Fig. 2).

Since a liquid media was used to introduce *Azospirillum* into lettuce seeds during imbibition, the germination enhancement due to presoaking already reported by several researchers in different crops (Ahmad et al., 1998; Jeyabal and Kuppaswamy, 1998) should not be discarded. Moreover, taking into account that both non-inoculated control and *Azospirillum*-inoculated seeds were imbibed with the same amount of phosphate buffer, the increase in germination percentage could be ascribed only to the bacterial action. In addition, presoaked, dried seeds could have higher germination percentages than those not dried after

imbibition. In this regard, the germination percentages in non-inoculated controls were 88.6 and 95.2 in non-dried (Fig. 1b) and dried (Fig. 2) seeds, respectively. However, even considering the possibility of a drying effect, the presence of *Azospirillum* significantly increased germination percentages at 0 mol m^{-3} NaCl in non-dried, non-stored lettuce seeds (Fig. 1b), and in dried seeds stored for 15 d (Fig. 2).

It has been shown in several crops and vegetables, that tolerance to salt at one growth stage is not correlated to tolerance at another stage (Shannon, 1997). On a similar ground, the results presented above could be evident only at the germination stage. In addition, lettuce production usually involves two main steps: (a) seed germination, and (b) seedlings transfer to seedbeds where plants could reach their commercial size. For these reasons, we performed an experiment where inoculated and non-inoculated seeds were germinated for 7 d in a non-saline media and plants irrigated with 0 , 30 , 50 , and 80 mol m^{-3} NaCl nutrient solutions during 30 additional days.

L. sativa responses to salt have been highly variable according to the cultivar (Shannon et al., 1983). However, some useful generalizations could be found in the literature regarding NaCl effects on this species. Lettuce yield is more affected by salt than broccoli, cucumber, spinach, cabbage and pepper, but less than carrots, onions and radish (Xu et al., 2000). High (60 mM) NaCl in nutrient solution strongly affected root elongation and mature vegetative growth of both spinach and lettuce, but especially in lettuce (Kaya et al., 2002). Even though NaCl did not significantly change fresh or dry weights, both ionic and osmotic effects of salt affected plant performance (Tarakcioglu and Inal, 2002). In general, NaCl significantly reduced weight gain at all the concentrations used in the present work. However, there were no significant differences in FW gain between plants exposed to 30 and 50 mol m^{-3} NaCl (Fig. 3a). In contrast, DW gain was progressively reduced with the increase in NaCl concentration (Fig. 3b).

Very little research has been carried out on the possible growth-promotion effects of *Azospirillum* spp. on lettuce (Agwah and Shahaby, 1993). This lack of information could be related to the apparent absence of the bacteria in the rhizoplane of the plant (Emtsev, 1994). In the present work and except at 50 mol m^{-3} NaCl, *Azospirillum*-inoculated plants had significantly higher FW gains than their respective non-inoculated controls after 30 d growth (Fig. 3a). Moreover, *Azospirillum*-inoculated plants grown at 30 mol m^{-3} NaCl had the same FW gain as non-inoculated controls at 0 mol m^{-3} NaCl (Fig. 3a). This growth-promoting effect of *Azospirillum* was more evident on the plant DW, where significant differences were found in all the treatments (Fig. 3b). Furthermore, *Azospirillum*-inoculated plants exposed to 50 and 80 mol m^{-3} NaCl had the same DW gains as non-inoculated controls exposed to 30 mol m^{-3} NaCl.

Since the marketable part of the lettuce plant is the aerial portion, it was interesting to study how NaCl affected BP and the effect of *Azospirillum* on such response. Shoot growth was affected more than root growth in lettuce exposed to low salinities (Shannon, 1997). In the present work, BP to the aerial

portion dropped significantly from 6.2 to 5.2 after 30 d growth at 80 mol m⁻³ NaCl (Fig. 4). Regarding the effects of *Azospirillum* under non-saline conditions BP to the aerial portion experienced a 56% increase in inoculated plants when compared to non-inoculated controls (Fig. 4). This effect was also evident at 80 mol m⁻³ NaCl, where *Azospirillum*-inoculated plants maintained the same BP as non-inoculated plants at 0 mol m⁻³ NaCl (Fig. 4).

The results presented here do not provide evidences on the mechanisms involved in salt stress relief at the tissue plant cell level. However, based on data published in relation to crops, several physiological changes induced by *Azospirillum* inoculation could explain its beneficial effects. In wheat seedlings, *A. brasilense* Sp245 inoculation partially reversed the negative effects produced by salt and osmotic stress on the relative elongation rate of shoots. Such reduction was accompanied by higher relative water content and water content (Creus et al., 1997). Moreover, higher water status in two inoculated cultivars exposed to osmotic stress, was related to a higher apoplastic water and cell wall elasticity (Creus et al., 1998). At the biochemical level, of all the alternatives thoroughly reviewed by Bashan et al. (2004) regarding the mode of action of *Azospirillum*, a strong possibility is the bacterial ability to produce or modify plant hormones, including gibberellins (GAs). These hormones play a key role in germination (Hilhorst and Toorop, 1997). Mature lettuce seeds contain relatively large amounts of abscisic acid (ABA) that can be limiting for germination. Upon imbibition ABA leaches out and germinability increases. The application of GAs also caused a decrease in ABA content that appeared to be metabolization rather than leaching (Hilhorst and Toorop, 1997). On the other hand, pre-sowing seed soaking treatments with gibberellic acid alleviated salt stress effects on seedlings of a salt-sensitive wheat cultivar (Datta et al., 1998).

Aside from producing or modifying phytohormones, *Azospirillum* could contribute to mitigate salt stress on plants through other mechanisms, each one acting alone or in combination (Bashan and Holguin, 1997). As an exopolysaccharide-producing bacteria, *Azospirillum* could restrict Na⁺ influx into roots (Ashraf et al., 2004). *Azospirillum* could also accumulate proline and glutamate in response to NaCl (Bashan and Holguin, 1997), and promote proline accumulation in maize exposed to water stress (Casanovas et al., 2002, 2003), thus acting as an osmoprotectant. Additionally, recent results involving NO production in *Azospirillum*-colonized tomato plants and the process of lateral root formation, add new insights to the complex scenery of plant–bacteria interactions (Creus et al., 2005).

In any case, as recently quoted by Bashan et al. (2004), almost no molecular studies regarding the effects of salt on *Azospirillum*–plant interactions have been carried out so far. Moreover, the same authors emphasized that the most fundamental omission in current knowledge is uncertainty on whether improved salt tolerance of the bacterium is needed to enhance the bacterium's effect on plants or if existing salt tolerance is adequate to ensure positive growth-promotion by inoculation (Bashan et al., 2004). The studies presented here

support the latter assumption and stimulate both basic and applied research tending to understand and improve lettuce production under saline conditions.

To conclude, inoculating imbibing seeds with *A. brasilense* Sp245 not only increased the germination percentages, but also plant growth and partition to the aerial portion in lettuce exposed to NaCl concentrations up to 80 mol m⁻³.

Acknowledgements

This work was supported by Agencia Nacional de Promoción Científica y Tecnológica, Consejo Nacional de Investigaciones Científicas y Técnicas and Universidad Nacional de Mar del Plata, Argentina. Authors thank Silvia A. Larraburu for her help at the lab, MSc Alicia L. Melegari for her valuable advice in the statistical analyses of data, and Prof. Ivone C. Barassi for editing the English text.

References

- Agwah, E.M.R., Shahaby, A.F., 1993. Associative effect of *Azospirillum* on vitamin C, chlorophyll content and growth of lettuce under field conditions. *Ann. Agric. Sci. Cairo* 38, 423–434.
- Ahmad, S., Anwar, M., Ullah, H., 1998. Wheat seed presoaking for improved germination. *J. Agron. Crop Sci.* 181, 125–127.
- Al-Karaki, G.N., 2001. Germination, sodium, and potassium concentrations of barley seeds as influenced by salinity. *J. Plant Nutr.* 24, 511–522.
- Alvarez, M.I., Sueldo, R.J., Barassi, C.A., 1996. Effect of *Azospirillum* on coleoptile growth in wheat seedlings under water stress. *Cer. Res. Commun.* 24, 101–107.
- Ashraf, M., Hasnain, S., Berge, O., Mahmood, T., 2004. Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. *Biol. Fert. Soils* 40, 157–162.
- Barassi, C.A., Creus, C.M., Casanovas, E.M., Sueldo, R.J., 2000. Could *Azospirillum* mitigate abiotic stress effects in plants? Auburn University web site available at: <http://www.ag.auburn.edu/argentina/pdfmanuscripts/barassi.pdf> (accessed 07/01/2001).
- Bashan, Y., Holguin, G., 1997. *Azospirillum*-plant relationships: environmental and physiological advances (1990–1996). *Can. J. Microbiol.* 43, 103–121.
- Bashan, Y., Holguin, G., de-Bashan, L.E., 2004. *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances (1997–2003). *Can. J. Microbiol.* 50, 521–577.
- Bacilio, M., Rodríguez, H., Moreno, M., Hernández, J.P., Bashan, Y., 2004. Mitigation of salt stress in wheat seedlings by a gfp-tagged *Azospirillum lipoferum*. *Biol. Fert. Soils* 40, 188–193.
- Casanovas, E.M., Barassi, C.A., Sueldo, R.J., 2000. *Azospirillum* inoculation of maize seed during imbibition. *Cer. Res. Commun.* 28, 25–32.
- Casanovas, E.M., Barassi, C.A., Sueldo, R.J., 2002. *Azospirillum* inoculation mitigates water stress effects in maize seedlings. *Cer. Res. Commun.* 30, 343–350.
- Casanovas, E.M., Barassi, C.A., Andrade, F.H., Sueldo, R.J., 2003. *Azospirillum*-inoculated maize plant responses to irrigation restraints imposed during flowering. *Cer. Res. Commun.* 31, 395–402.
- Coons, J.M., Kuehl, R.O., Simons, N.R., 1990. Tolerance of 10 lettuce cultivars to high temperature combined with NaCl during germination. *J. Am. Soc. Hortic. Sci.* 115, 1004–1007.
- Creus, C.M., Sueldo, R.J., Barassi, C.A., 1996. *Azospirillum* inoculation in pre-germinating wheat seeds. *Can. J. Microbiol.* 42, 83–86.
- Creus, C.M., Sueldo, R.J., Barassi, C.A., 1997. Shoot growth and water status in *Azospirillum*-inoculated wheat seedlings grown under osmotic and salt stresses. *Plant Physiol. Biochem.* 35, 939–944.
- Creus, C.M., Sueldo, R.J., Barassi, C.A., 1998. Water relations in *Azospirillum*-inoculated wheat seedlings under osmotic stress. *Can. J. Bot.* 76, 238–244.

- Creus, C.M., Sueldo, R.J., Barassi, C.A., 2004. Water relations and yield in *Azospirillum*-inoculated wheat exposed to drought in the field. *Can. J. Bot.* 82, 273–281.
- Creus, C.M., Graziano, M., Casanovas, E.M., Pereyra, M.A., Simontacchi, M., Puntarulo, S., Barassi, C.A., Lamattina, L., 2005. Nitric oxide is involved in the *Azospirillum brasilense*-induced lateral root formation in tomato. *Planta* 221, 297–303.
- Datta, K.S., Varma, S.K., Angrish, R., Kumar, B., Kumari, P., 1998. Alleviation of salt stress by plant growth regulators in *Triticum aestivum* L. *Biol. Plant.* 40, 269–275.
- Döbereiner, J., Day, J.M., 1976. Associative symbiosis in tropical grasses: characterization of microorganisms and dinitrogen fixing sites. In: Newton, W.E., Nyman, C.J. (Eds.), *Proceedings of the First International Symposium on Nitrogen Fixation*. Washington State University Press, Pullman, pp. 518–538.
- Emtsev, V.T., 1994. Associative symbiosis of soil diazotrophic bacteria and plants and its contribution to yields of vegetables. *Pochvovedenie (Russian Fed.)* 4, 74–84.
- Graifenberg, A., Lipucci di Paola, M., Giustiniani, L., 1993. Yield and growth of globe artichoke under saline-sodic conditions. *HortScience* 28, 791–793.
- Hamaoui, B., Abbadi, J.M., Burdman, S., Rashid, A., Sarig, S., Okon, Y., 2001. Effects of inoculation with *Azospirillum brasilense* on chickpeas (*Cicer arietinum*) and faba beans (*Vicia faba*) under different growth conditions. *Agronomie* 21, 553–560.
- Hamdi, H., 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in arid climate. *Microbiol. Mol. Biol. R* 63, 968–989.
- Hartmann, A., 1988. Osmoregulatory properties of *Azospirillum* spp. In: Klingmüller, W. (Ed.), *Azospirillum IV: Genetics, Physiology, Ecology*. Springer-Verlag, Berlin, pp. 122–130.
- Hilhorst, H.W.M., Toorop, P.E., 1997. Review on dormancy, germinability, and germination in crop and weed seeds. *Adv. Agron.* 61, 111–165.
- International Seed Testing Association, 1999. *The germination test*. In: *Proceedings of the International Seed Testing Association*, International Seed Testing Association Press, Zurich, pp. 155–199.
- Jeyabal, A., Kuppuswamy, G., 1998. Effect of seed soaking on seedling vigour, growth and yield of rice. *J. Agron. Crop Sci.* 180, 181–190.
- Kaya, C., Higgs, D., Sakar, E., 2002. Response of two leafy vegetables grown at high salinity to supplementary potassium and phosphorus during different growth stages. *J. Plant Nutr.* 25, 2663–2676.
- Kotb, T.H.S., Watanabe, T., Ogino, Y., Tanji, K.K., 2000. Soil salinization in the Nile Delta and related policy issues in Egypt. *Agric. Water Manage.* 43, 239–261.
- Martínez, V., Bernstein, N., Läuchli, A., 1996. Salt-induced inhibition of phosphorus transport in lettuce plants. *Physiol. Plantarum* 97, 118–122.
- Odegaro, O.A., Smith, O.E., 1969. Effect of kinetin, salt concentration, and temperature on germination and early seedling growth of *Lactuca sativa* L. *J. Am. Soc. Hortic. Sci.* 94, 167–170.
- Okon, Y., 1985. *Azospirillum* as a potential inoculant for agriculture. *Trends Biotechnol.* 3, 223–228.
- Okon, Y., Albrecht, S.L., Burris, H., 1977. Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. *Appl. Environ. Microb.* 33, 85–88.
- Pardossi, A., Bagnoli, G., Malorgio, F., Campiotti, C.A., Tognoni, F., 1999. NaCl effects on celery (*Apium graveolens* L.) grown in NFT. *Sci. Hortic.* 81, 229–242.
- Postgate, J.R., 1969. Viable counts and viability. In: Norris, J.R., Ribbons, D.W. (Eds.), *Methods in Microbiology*. Academic Press, New York, pp. 611–628.
- Qadir, M., Ghafoor, A., Murtaza, G., 2000. Amelioration strategies for saline soils: a review. *Land Degrad. Dev.* 11, 501–521.
- Rehman, S., Harris, P.J.C., Bourne, W.F., 1999. Effect of artificial ageing on the germination, ion leakage and salinity tolerance of *Acacia tortilis* and *A. coriacea* sees. *Seed Sci. Technol.* 27, 141–149.
- Rodríguez Cáceres, E.A., 1982. Improved medium for isolation of *Azospirillum* spp. *Appl. Environ. Microb.* 44, 990–991.
- SAS Institute, 2000. *The SAS OnlineDoc system for Windows*. Release 8.2. SAS Institute Inc., Cary, New Carolina.
- Tarakcioglu, C., Inal, A., 2002. Changes induced by salinity, demarcating specific ion ratio (Na/Cl) and osmolality in ion and proline accumulation, nitrate reductase activity, and growth performance of lettuce. *J. Plant Nutr.* 25, 27–41.
- Shaner, G., Finney, R.E., 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67, 1051–1056.
- Shannon, M.C., McCreight, C., Draper, J.H., 1983. Screening tests for salt tolerance in lettuce. *J. Am. Soc. Hortic. Sci.* 108, 225–230.
- Shannon, M.C., 1997. Adaptation of plants to salinity. *Adv. Agron.* 60, 75–120.
- Xu, G., Magen, M., Tarchitzky, J., Kafkafi, U., 2000. Advances in chloride nutrition of plants. *Adv. Agron.* 68, 97–150.
- Zahir, Z.A., Arshad, M., Frankenberger Jr., W.T., 2004. Plant growth promoting rhizobacteria: applications and perspectives in agriculture. *Adv. Agron.* 81, 97–168.