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ORIGINAL ARTICLES

Restoration of Growth of Durum Wheat (*Triticum durum* var. waha) Under Saline Conditions Due to Inoculation with the Rhizosphere Bacterium *Azospirillum brasilense* NH and Extracts of the Marine Alga *Ulva lactuca*

Elhafid Nabti · Mohamed Sahnoune · Mostefa Ghoul · Doreen Fischer · Andreas Hofmann · Michael Rothballer · Michael Schmid · Anton Hartmann

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Abstract Inoculation with the rhizosphere bacterium *Azospirillum brasilense* NH, originally isolated from salt-affected soil in northern Algeria, greatly enhanced growth of durum wheat (*Triticum durum* var. waha) under saline soil conditions. Important plant parameters like the rate of germination, stem height, spike length, dry weight of roots and shoots, chlorophyll a and b content, proline and total sugar contents, 1000-seed weight, seed number per spike, and weight of seeds per spike were measured. At salt stress conditions (160 and 200 mM NaCl) *A. brasilense* NH restored almost completely vegetative growth and seed production. The combination with extracts of the marine alga *Ulva lactuca* resulted in even more improved salt tolerance of durum wheat. Proline and total sugar accumulation, a sign of physiological plant stress under inhibitory salt conditions, was reduced in plants inoculated with *A. brasilense* NH with and without addition of algal extracts. Inoculation with the salt-sensitive *A. brasilense* strain Sp7 could not restore salt-affected plant growth at 200 mM NaCl. Furthermore, it could be demonstrated by fluorescence in situ hybridization and confocal laser scanning microscopy that *A. brasilense* NH is able to colonize roots of durum wheat endophytically under salt-stressed conditions. Thus, the salt-tolerant rhizobacterium *A. brasilense* NH could effectively provide alone or in combination with extracts of *U. lactuca* a promising solution to overcome salt inhibition which is a major threat hindering productive wheat cultivation in arid saline soils.

Keywords *Azospirillum brasilense* · Wheat · Salinity stress · Salt-affected soil · Plant growth promotion · Endophytic colonization · Confocal laser scanning microscopy

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E. Nabti
Department of Microbiology, Laboratory of Applied Microbiology, Faculty of Nature and Life Sciences, University of Bejaïa, Targa Ouzemmour, 06000 Bejaïa, Algeria
e-mail: elhnabti1977@yahoo.fr

M. Sahnoune
Department of Biology of Organisms and Populations, Laboratory of Ecology and Environment, Faculty of Nature and Life Sciences, University of Bejaïa, Targa Ouzemmour, 06000 Bejaïa, Algeria
e-mail: sahnounemohamed@yahoo.fr

M. Ghoul
Department of Biology, Laboratory of Bacterial Ecology, Faculty of Biology, University of Setif, 19000 Setif, Algeria
e-mail: ghoulmostefa@yahoo.fr

D. Fischer · A. Hofmann · M. Rothballer · M. Schmid · A. Hartmann (✉)
Department Microbe-Plant Interactions, German Research Center for Environmental Health (GmbH), Helmholtz Zentrum München, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany
e-mail: anton.hartmann@helmholtz-muenchen.de

D. Fischer
e-mail: doreen.fischer@helmholtz-muenchen.de

A. Hofmann
e-mail: andreas.hofmann@helmholtz-muenchen.de

M. Rothballer
e-mail: rothballer@helmholtz-muenchen.de

M. Schmid
e-mail: michael.schmid@helmholtz-muenchen.de

Introduction

Soil salinity constitutes a major obstacle for agriculture in arid and semiarid regions. Actually, 20% of the world's cultivated area and almost half of the world's irrigated soils are severely affected by the lack of water and increased salinity and are thus lost for agriculture. Many of these areas are confined to the tropical and subtropical Mediterranean areas (Carpita 1985; Cordovilla and others 1994; Zahran 1999). To solve these problems, improvement of management practices by, for example, using suitable plant cultivars or osmotolerant plant-growth-promoting rhizobacterial strains (PGPR) (for example, *Pseudomonas* and *Azospirillum*) are being developed (Creus and others 1998; Mayak and others 2004; Bartels and Sunkar 2005; Egamberdiyeva 2005). In addition, supplements with natural substances (that is, extracts of marine alga) were considered (Ghoul 1990), allowing the restoration and improvement of salt-affected crop growth.

Cytoplasmic membranes of organisms are permeable to water but not to other metabolites. Therefore, hyper- or hypo-osmotic shock exerted on cells causes a concomitant decrease or increase in the cytoplasmic volume leading to plasmolysis. The exposure of bacteria to high osmolarity conditions decreases water activity in their cytoplasm (Epstein 1986) and most of the cellular proteins and other biological macromolecules as well as essential functions are impaired (Bakker and others 1987). Also, sudden plasmolysis inhibits various physiological processes, ranging from energy and nutrient uptake to inhibition of DNA replication and macromolecule biosynthesis (Kogut and Russell 1987; Bartels and Sunkar 2005).

High salinity constitutes an environmental stress also for rhizospheric bacteria. In addition to general effects outlined above, alteration of proteins involved in the initial attachment steps (adsorption and anchoring) of bacteria to plant roots in symbiotic interaction occur as well as inhibition of bacterial nodulation and nitrogen fixation activity, alteration of exopolysaccharide (EPS) and lipopolysaccharide (LPS) composition of the bacterial cell surface, impairment of molecular signal exchange between bacteria and their plant host due to the alteration of membrane glucan contents, and inhibition of bacterial mobility and chemotaxis toward plant roots. High salinity decreases bacterial numbers colonizing root cells endophytically because plants utilize proline, proline betaine, and glutamate under osmotic stress conditions and thus deprive rhizobacteria of these substances as energy and carbon sources as well as osmolytes which finally limits their growth. Depletion of potassium ions by plants reduces the ability of rhizobacteria to use potassium ions as a primary osmoregulator (Bakker and others 1987; Phillips and others 1992; Jofré and others 1998).

Many studies report that osmotic stress distorts a plant's physiology and cellular structure (Bartels and Sunkar 2005). High salinity causes an inhibition and deterioration of several stages of plant life: germination, synthesis of phytohormones and other plant-growth-stimulating factors, photosynthesis, maturation of cell walls, plant morphology and elaboration, root and stem growth, ionic transport and nutrient uptake, and general enzymatic activity (Xiong and Zhu 2002).

Plant-growth-promoting rhizobacteria (PGPR) have a high potential for agriculture because they can improve plant growth, especially under limiting or stress conditions. For example, bacteria of the species *Azospirillum brasilense* were successfully applied as an inoculant in agriculture especially with Gramineae resulting in improved yields under severe conditions (Okon and Kapulnik 1986; Okon and Labandera-Gonzalez 1994; Okon and Vanderleyden 1997; Dobbelaere and others 2001; Hartmann and Baldani 2006). For *Azospirillum*, this growth promotion resulted from bacterial phytohormone synthesis, which promotes root development and the uptake of nutrients and water (Bashan and Holguin 1997; Dobbelaere and others 2003; Perrig and others 2007). Water stress of plants was shown to be particularly diminished in wheat seedlings by inoculation with *Azospirillum brasilense* Sp245 (Creus and others 1998). Recently, an *A. brasilense* strain NH was isolated from salt-affected soil in northern Algeria (Nabti and others 2007). We reported the phylogenetic position and its most interesting halotolerant and halophilic properties, which exceeded the halotolerance of other reported halotolerant *A. brasilense* strains and other PGPR. Although its growth was retarded at low salt concentrations, its optimal growth was at 200 mM NaCl in minimal medium without osmoprotective additives (Nabti and others 2007). *Azospirillum brasilense* NH produces the auxin indole acetic acid (IAA) in osmotic stress conditions, while osmosensitive *A. brasilense* Sp7 showed only marginal IAA production. Using glycine betaine and extracts of *Ulva lactuca*, its growth could be stimulated even at 300–500 mM NaCl (Nabti and others 2007).

Aqueous extracts of *Ulva lactuca* constitute an efficient source of osmoprotectants for microorganisms under saline stress. This alga contains high levels of various betaines, amino acids, proteins, and dimethylsulfoniopropionate (DMSP) (Ghoul 1990; Ghoul and others 1995). DMSP was demonstrated to be a potent osmoprotectant in several bacteria such as *E. coli* (Ghoul and others 1995), *Salmonella thyphimurium* (Galinski 1995), *Sinorhizobium meliloti* (Pichereau and others 1998), and 40% of marine prokaryotes (Malmstrom and others 2004). DMSP is also an efficient osmoprotectant for agricultural bacteria (Reed 1983; Rhodes and Hanson 1993). Although the effect of salinity on plant growth was exhaustively studied (Saqib and others

2004; Sepaskhah and others 2006), only few data are yet available on the combined effects of osmotolerant *A. brasilense* strains and natural osmoprotectants (for example, marine alga) on plant growth under saline stress in the field. The ability of *A. brasilense* NH to promote the growth of wheat under salinity stress was reported recently for only two basic growth parameters (Nabti and others 2007).

To determine the impact of *A. brasilense* NH and/or of aqueous extracts of *U. lactuca* on the restoration and improvement of the growth of durum wheat (*Triticum durum*, var. waha), a detailed study of plant performance was carried out in pots and field experiments under saline conditions. In addition, a laboratory monoxenic inoculation study with durum wheat and *A. brasilense* NH was carried out under saline and nonsaline conditions to reveal the mode of root colonization (potential for endophytic colonization) of *A. brasilense* NH using fluorescence in situ hybridization (FISH) analysis combined with confocal laser scanning microscopy (CLSM).

Materials and Methods

Plant and Bacteria Used

Durum wheat (*Triticum durum* var. waha), the predominantly used cultivar of wheat in the Bejaïa area of northern Algeria, was studied (INRA, Bejaïa, Algeria). The salt-tolerant bacterium *A. brasilense* NH, isolated from the salt-affected soil of a wheat field in the Bejaïa area (Nabti and others 2007), was applied as an inoculum. The salt-sensitive strain *A. brasilense* Sp7 was used as a control. *Burkholderia phytofirmans* PSJN was a gift from Dr. Angela Sessitsch (Seibersdorf, Austria).

Preparation of Algal Aqueous Extract

About 5 kg of green alga *Ulva lactuca* was harvested from the Gulf of Bejaïa (Algeria) in March 2006. After washing with clean seawater, distilled water was added to make 500 g fresh weight of alga per liter. To release the osmoprotective substances, the alga aqueous suspension was autoclaved at 110°C for 30 min. The extracts had the following nutritive composition (g/100 g dry matter): glucids, 36; lipids, 0.8; protids, 17; and minerals (K, P, N, and Fe), 30 (Ghoul 1990; Cayla 1995).

Characteristics of the Experimental Site and Soil

The site of the experiment (Ihaddadhen, Bejaïa, Algeria) was at 500 m altitude and oriented north toward the sea. Chemical characteristics of the soil were as follows: organic matter, 3.1%; total N, 0.48%; mineral N, 0.025%; phosphate, 0.1%; potassium, 0.13%; Ca^{2+}, 0.45%; Mg^{2+}, 0.11%; water-holding capacity, 25%; pH 6.4; and 0.85% salinity.

Experimental Design and Plant Culture

Three experiments were conducted simultaneously from March to June 2006 (that is, from sowing to complete plant maturity the 12th week after sowing) under a natural light/dark regime and temperatures ranging from 25 to 32°C during the time period. Pot experiment 1 and pot experiment 2 were performed at an initial NaCl concentration of 100 and 150 mM, respectively. In the third week after sowing the salinity was increased to a final concentration of 160 and 200 mM NaCl, respectively. Each pot experiment consisted of ten treatments (T1–T10) with 50 plants each (with finally 1 plant per pot); pot experiment 1 and pot experiment 2 had six different treatments (T3–T8), where NaCl was involved (Table 1). Pots (1.5 l) were filled with autoclaved soil (110°C for 30 min) taken from the experimental site described above. Surface disinfection of seeds was performed by immersion in 1% NaOCl solution followed by thorough washing in distilled water. Five surface-disinfected seeds were sown per pot; they were put together 3 cm deep into the soil at the center of the pot. Algal

Table 1 Composition and codes of the ten treatments (T1–T10) used in pot experiment 1, pot experiment 2, and the field experiment

Code	Treatment composition	Abbreviation
T1	Soil + Wheat (Control)	Control
T2	Soil + Wheat + *Ulva lactuca* aqueous extracts	U
T3	Soil + Wheat + NaCl[a]	NaCl
T4	Soil + Wheat + NaCl[a] + *U. lactuca* aqueous extracts	NaCl + U
T5	Soil + Wheat + NaCl[a] + *A. brasilense* NH	NaCl + NH
T6	Soil + Wheat + NaCl[a] + *A. brasilense* Sp7	NaCl + Sp7
T7	Soil + Wheat + NaCl[a] + *U. lactuca* aqueous extracts + *A. brasilense* NH	NaCl + U + NH
T8	Soil + Wheat + NaCl[a] + *U. lactuca* aqueous extracts + *A. brasilense* Sp7	NaCl + U + Sp7
T9	Soil + Wheat + *A. brasilense* Sp7	Sp7
T10	Soil + Wheat + *A. brasilense* NH	NH

[a] Pot expt. 1 (160 mM NaCl); Pot expt. 2 and Field expt. (200 mM NaCl)

aqueous extracts (100 ml, 500 g/l) and/or 10 ml of bacterial suspension (10^6 CFU/ml) were added to the appropriate seeds immediately after sowing. No fertilizer was applied throughout the entire experiment. After emergence of the first leaf, plant density was reduced to one plantlet per pot. The positions of the 800 pots of the two pot experiments were completely randomized to exclude any location effect on plant growth. The total randomization was carried out using the STAT-ITCF software (STAT-ITCF 1992). To avoid cross-contamination, the pots were placed 30 cm apart and the soil surface was covered with a 3-cm-thick layer of sterile vermiculite. Pots were wrapped in black plastic to mitigate the effects of light. All potted plants were isolated from precipitation water by being in a greenhouse.

The area of the field experiment was 225 m^2. The cultivation procedure was the same as used in the pot experiments, except that the seeds were placed in 5-cm-deep holes in the soil surface. For each treatment 30 plants were sown in a single line with 80 cm between plants and 75 cm between lines. The soil surface was not covered with vermiculite. Altogether, the field experiment consisted of 300 nonrandomized plants.

Plant Irrigation

For all three experiments, each pot (pot experiment 1 and pot experiment 2) or a hole in the ground (field experiment) was irrigated once a week with 500 ml of nonsaline water from a nearby well. In the first 3 weeks, the pots or holes in the ground in which salt was involved were irrigated with 500 ml of 100 mM NaCl (pot experiment 1) or 150 mM NaCl (pot experiment 2 and field experiment) instead of water. At the third week after sowing for all three experiments the initial NaCl concentrations were increased from 100 and 150 mM to 160 and 200 mM NaCl, respectively. The salinity in the treatments to which salt was not added was about 1.5 mM NaCl. The effective salt concentrations were measured using a conductivity meter. The cultures progressed until complete plant maturity (12th week after sowing).

Measured Plant Parameters

The 50 and 30 sets of five seeds per treatment described above for the pot and field experiments, respectively, were used to determine the germination rates, expressed as confidence intervals ($P = 0.05$) of mean numbers of germinations (M ± 2 SE) observed over the first 9 days after sowing. In addition, 17 other parameters were measured on plants (from 9th to 12th week after sowing). These parameters (indicated by the superscript numbers) are listed as follows: [1]stem height (cm), [2]length (cm) and [3]width (mm) of the largest leaf, [4]stem dry weight (g), [5]root dry weight (g), [6]total spike length (beard hairs included) (cm), [7]beard hair length (cm), [8]spike length (beard hairs not included) (cm), [9]spike dry weight (g), [10]seed number per spike, [11]total seed weight per spike, [12]1000-seeds weight (g), and on mature green leaves, [13]chlorophyll a, [14]chlorophyll b, and [15]total chlorophyll rates/fresh matter (/FM) (mg/g), [16]proline content/FM (µg/g) (Bengston and others 1978), and [17]total sugars rate/FM (µg/g) (Anthron method). Parameters 1–12 were measured of all experimental plants, but chlorophylls, proline, and sugar contents (parameters 13–17) were determined only for ten plants per treatment.

Statistical Analysis

The values of the measured parameters are expressed as mean ± standard deviation (M ± SD) or mean ± 1.96(2) standard error (M ± 2SE). For statistical comparison of these mean values, one-way ANOVA and Fisher's least significant difference (LSD) tests were applied ($P = 0.05$). The ten treatments within each experiment and the three experiments within each treatment were compared for all the measured parameters separately (monofactorial comparison). For multifactorial comparison, principal component analysis (PCA) was used to display the correlations between the various plant parameters and the affinities between the different treatments within and between experiments. For this, a PCA was performed for each experiment separately and then another PCA was done for all three experiments taken together. The position of the treatments in the PCA projections were also tested using ANOVA and Fisher's LSD tests ($P = 0.05$). The programs used for all the statistical analyses were from the Statistica 7.0 package (STAT-ITCF 1992). PCA analyses included ten plants per treatment and all 17 measured parameters.

In Situ Localization of *A. brasilense* NH in/on Wheat Roots with FISH Analysis and CLSM in a Monoxenic System

Wheat Seed Sterilization

Thirty wheat seeds were shaken vigorously for 2 min in ethanol (70% v/v) and then treated for 15 min with a NaOCl (2%) solution, washed several times with sterile distilled water, and finally treated for 1 h in a solution with a mixture of antibiotics (penicillin 600 mg/ml, streptomycin 250 mg/ml, cycloheximide 100 mg/ml). Finally, seeds were germinated on nutrient broth plates at 30°C in the dark to display possible contamination (Aßmus and others 1995).

Inoculum Preparation

Liquid nutrient broth (NB) medium was inoculated with *A. brasilense* NH and incubated overnight with shaking at 30°C. The culture was centrifuged at 6,000*g* for 5 min and the pellet was washed twice with 20 ml of sterile PBS (phosphate-buffered saline). The bacterial suspension used for the experiment was 10^8 cells/ml ≈OD 0.7 at 436 nm.

Monoxenic Plant Growth System

Test tubes (200-ml volume) were filled to one-third of their volume with quartz sand and 10 ml of the plant nutrient solution Length Ashton (Sigma, St. Louis, MO) (1 g/100 ml) and autoclaved at 121°C for 20 min. NaCl was added to the appropriate tubes at 200 mM concentration. Wheat seedlings, germinated under sterile conditions, were washed with sterile distilled water and incubated with the bacterial suspension for 1 h at room temperature. In each tube, one seed was planted at a depth of 1 cm. The glass tubes were exposed to daylight for growth for 30 days at room temperature.

Preparation of Root Samples

After 30 days, seedlings were carefully removed from the tubes and washed by shaking in sterile PBS. Root pieces 15–30 mm in length from all parts of the root system were transferred into the fixation buffer (paraformaldehyde 4% in PBS) and incubated at room temperature for 2 h. Samples were washed and dehydrated by incubating for 5 min each in an increasing ethanol series at 50, 80, and 96%. Each root piece was immobilized on gelatin-coated slides with a droplet of agarose (0.25%) on both ends.

In Situ Hybridization and Microscopy

The following oligonucleotide probes were used: Eub-338-I (Amann and others 1990), II, and III (Daims and others 1999) as equimolar mixture labeled with the fluorescent dye fluorescein (FITC), complementary to a region of the 16S rRNA specific for the domain Bacteria, and Abras-1420-Cy3, specific for the species *A. brasilense* (Stoffels and others 2001). Coated slides already prepared for hybridization were used. Each spot received 15 µl of hybridization buffer [(NaCl (0.9 M), 20 mM, Tris–HCl [pH 7.2], SDS (0.01%), EDTA (5 mM, deionized formamide (20%)]. A total of 2 µl of the probes was added. Incubation was performed at 46°C for 2 h. The slides were transferred to the washing buffer (20 mM Tris; 0.01% SDS; 5 mM EDTA; 0.09 M NaCl) and then incubated at 48°C for 20 min. Finally, the slides were rinsed with bi-distilled water and dried in an air stream.

Fluorescing cells on or in roots were detected by a confocal laser scanning microscope (LSM-510 MEta, Zeiss, Oberkochen, Germany). An argon ion laser supplied the wavelength of 488 nm for excitation of FITC, and two helium neon lasers provided excitation wavelengths of 543 and 633 nm for Cy3 and Cy5 excitation, respectively. A water immersion lens (C-Apochromat 63×/1.2 W, Zeiss, Oberkochen, Germany) was used for observation.

Detection of ACC Deaminase Activity

The 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity of bacteria was detected according to the method of Brown and Dilworth (1975) using *Burkholderia phytofirmans* PSJN as positive reference culture.

Results

Effects of Various Treatments on Germination Under Saline and Nonsaline Conditions

The germination rates of wheat seeds were greatly influenced by salt stress (Table 2). They were either inhibited by salt or restored partially to completely following the experimental treatments (Fig. 1). In the presence of 160 mM NaCl (treatment 3) the germination rate was almost completely inhibited. The inoculation with *A. brasilense* NH together with the application of algal extracts (treatment 7) resulted in the same high numbers of germinated seeds compared to the control without salt stress (treatment 1). The addition of algal extracts or *A. brasilense* NH alone (treatments 4 and 5) yielded only partial recovery. The inoculation with *A. brasilense* Sp7 reduced the NaCl effect only very slightly (treatment 6, Fig. 1a).

When 200 mM NaCl was applied in pot and field experiments, the germination rate was completely inhibited and could not be restored by inoculation with *A. brasilense* Sp7 (treatments 3 and 6, Fig. 1b, c). The inoculation with *A. brasilense* NH (treatment 5) restored the germination only partly and supported the germination of about one-third of the seeds. The combination of inoculation with the strain NH and treatment with *U. lactuca* extract promoted the germination of all seeds (treatment 7), although with a delay of about 4 days compared with the control treatments without salt stress (Fig. 1c). On the other hand, wheat seeds inoculated with *A. brasilense* Sp7 were still greatly affected by salt stress and Sp7 did not reduce the stress effect on germination at 200 mM NaCl (treatments 4 and 8, Fig. 1b, c).

Table 2 Germination rates (as mean numbers of germinations ± standard deviation) of wheat seeds over the first 9 days after sowing

Code	Treatment	Day 3[1]			Day 5		
		Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)	Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)
T1	Control	4.82 ± 0.59^{Ga}	4.82 ± 0.59^{Da}	4.82 ± 0.59^{DEa}	4.98 ± 0.14^{Ga}	4.98 ± 0.14^{Fa}	4.98 ± 0.14^{Ea}
T2	U	4.92 ± 0.27^{Ga}	4.92 ± 0.27^{Da}	4.92 ± 0.27^{Ea}	4.92 ± 0.27^{Ga}	4.92 ± 0.27^{Fa}	4.92 ± 0.27^{Ea}
T3	NaCl	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	0.02 ± 0.14^{Aa}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T4	NaCl + U	2.80 ± 0.53^{Db}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	4.02 ± 0.38^{Dc}	0.42 ± 0.49^{Ba}	0.28 ± 0.45^{Cb}
T5	NaCl + NH	1.08 ± 0.44^{Bb}	0.84 ± 0.37^{Ba}	0.84 ± 0.37^{Cc}	4.24 ± 0.72^{Ec}	1.86 ± 0.35^{Cb}	1.80 ± 0.40^{DEa}
T6	NaCl + Sp7	0.10 ± 0.30^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	0.48 ± 0.50^{Bb}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Ac}
T7	NaCl + NH + U	3.96 ± 0.35^{Eb}	0.90 ± 0.36^{Ba}	0.90 ± 0.36^{Bc}	4.98 ± 0.14^{Gb}	4.86 ± 0.35^{Da}	4.16 ± 0.55^{Cc}
T8	NaCl + Sp7 + U	2.00 ± 0.40^{Cb}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	2.94 ± 0.24^{Cb}	0.56 ± 0.50^{Ba}	0.32 ± 0.47^{Ba}
T9	Sp7	4.80 ± 0.67^{Ga}	4.80 ± 0.67^{Da}	4.80 ± 0.67^{DEa}	4.84 ± 0.65^{FGa}	4.84 ± 0.65^{EFa}	4.84 ± 0.65^{Da}
T10	NH	4.50 ± 0.88^{Fb}	4.50 ± 0.89^{Cb}	4.50 ± 0.88^{Daa}	4.74 ± 0.66^{Fa}	4.74 ± 0.66^{Ea}	4.74 ± 0.66^{DEa}

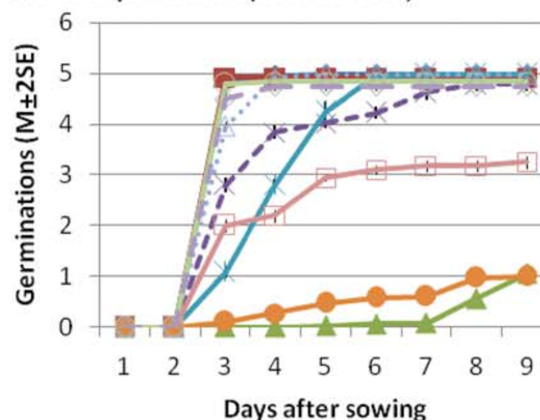
Code	Treatment	Day 7			Day 9		
		Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)	Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)
T1	Control	4.98 ± 0.14^{Fa}	4.98 ± 0.14^{Ea}	4.98 ± 0.14^{Ea}	4.98 ± 0.14^{Fa}	4.98 ± 0.14^{Ea}	4.98 ± 0.14^{Da}
T2	U	4.92 ± 0.27^{Fa}	4.92 ± 0.27^{Ea}	4.92 ± 0.27^{Ea}	4.92 ± 0.27^{EFa}	4.92 ± 0.27^{Ea}	4.92 ± 0.27^{Da}
T3	NaCl	0.08 ± 0.27^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	0.12 ± 0.33^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T4	NaCl + U	4.62 ± 0.49^{Db}	0.54 ± 0.50^{Ba}	0.54 ± 0.50^{Ec}	4.78 ± 0.42^{DEb}	0.58 ± 0.49^{Ba}	0.58 ± 0.49^{CDb}
T5	NaCl + NH	4.98 ± 0.14^{Fa}	1.92 ± 0.27^{Ca}	1.92 ± 0.27^{DEa}	4.98 ± 0.14^{Fa}	1.92 ± 0.27^{Ca}	1.92 ± 0.27^{CDa}
T6	NaCl + Sp7	0.60 ± 0.49^{Bb}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Ac}	0.84 ± 0.37^{Bb}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Ac}
T7	NaCl + NH + U	4.98 ± 0.14^{Fb}	4.92 ± 0.27^{Ea}	4.92 ± 0.27^{Eb}	4.98 ± 0.14^{Fb}	4.92 ± 0.27^{Ea}	4.92 ± 0.27^{Db}
T8	NaCl + Sp7 + U	3.18 ± 0.39^{Cb}	0.60 ± 0.49^{Ba}	0.60 ± 0.49^{Ba}	3.26 ± 0.44^{Cb}	0.62 ± 0.49^{Ba}	0.62 ± 0.49^{Ba}
T9	Sp7	4.84 ± 0.65^{EFa}	4.84 ± 0.65^{DEa}	4.84 ± 0.65^{BCa}	4.84 ± 0.65^{DEFa}	4.84 ± 0.65^{DEa}	4.84 ± 0.65^{Ba}
T10	NH	4.74 ± 0.66^{DEa}	4.74 ± 0.66^{Da}	$4.74 \pm 0.66C^{Da}$	4.74 ± 0.66^{Da}	4.74 ± 0.66^{Da}	4.74 ± 0.66^{BCa}

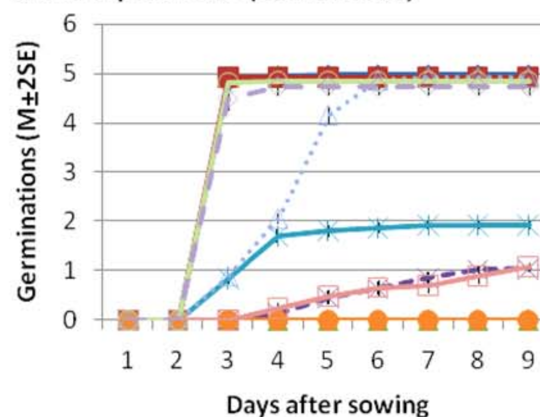
The mean values were determined on 50 and 30 sets of five seeds for pot experiments and field experiment, respectively. A–H and a–c express the statistical evaluation and compare the ten treatments (plots) (1–10) within each experiment (columns) and the three experiments within the same treatment (rows), respectively. Values accompanied by the same letter are not significantly different according to Fisher's least significant difference test ($P < 0.05$)

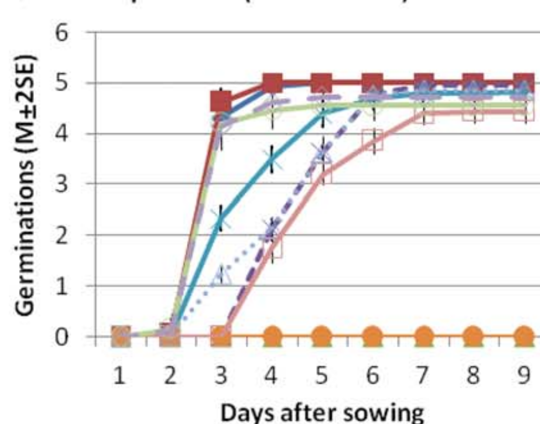
[1] The germinations began only on the third day after sowing for all the three experiments

Effects of the Various Treatments on Growth and Yield Parameters Under Saline and Nonsaline Conditions

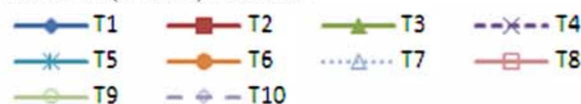
Under nonsaline conditions (treatments 1, 2, 9, and 10), the changes observed are small but significant in some cases. Stem height ([1]SH) increased slightly in the presence of the algal extracts (treatment 2) and in the presence of *Azospirillum brasilense* Sp7 (treatment 9) and NH (treatment 10) compared to the control without bacterial inoculation (treatment 1) (Table 3). The stimulatory effect of both *A. brasilense* Sp7 and NH inoculation (treatments 9 and 10) on leaf growth [leaf length ([2]LL) and leaf width ([3]LW)] was more significant than the effect of algal extracts (treatment 2). Stem dry weight ([4]ADW) was more improved by the addition of algal extracts than by inoculation with Sp7 or NH; the control plants always presented the lowest [4]ADW value. Root dry weight ([5]RDW) was more stimulated by inoculation with Sp7 and NH than by the addition of the algal extracts. Total spike length ([6]TSL) was increased in plants treated with algal extracts (treatment 2) as well as in the inoculated plants (treatments 9 and 10) compared to the control. The spike length (without hairs) ([8]SL) and spike hair length alone ([7]HL) showed effects similar to those of [6]TSL. The spike dry weight ([9]SDW) increased only slightly in the presence of algal extracts and the bacterial inocula. In the pot experiments, inoculation with strain Sp7 promoted [9]SDW slightly more than with the NH strain. The number of seeds per spike ([10]SNS) was affected only in the presence of algal extracts, whereas inoculation with Sp7 or NH did not result in any difference from the control. In contrast, total weight of seeds per spike ([11]SWS) in the pot experiments increased







Treatments (T1-T10) markers:



The formulae of the 10 treatments: **T1:** Control (Wheat alone); **T2:** U; **T3:** NaCl; **T4:** NaCl + U; **T5:** NaCl + NH; **T6:** NaCl + Sp7; **T7:** NaCl + U + NH ; **T8:** NaCl + U + Sp7; **T9:** Sp7; **T10:** NH (See also Table 1 for detailed information).

Fig. 1 Germination rates (as mean numbers of germinations ± 2 standard errors) of wheat seeds in the first 9 days after sowing. The mean values were determined on 50 and 30 sets of five seeds in the pot experiments (**a**, **b**) and the field experiment (**c**), respectively (each point represents the cumulated mean number of germinated seeds among the five seeds at the corresponding day). The values with nonoverlapping confidence intervals (M ± 2SE) are significantly different ($P = 0.05$)

after the addition of algal extracts and inoculation with *A. brasilense* Sp7, whereas *A. brasilense* NH was less efficient in this case. In the pot experiments, the most important yield parameter, 1000-seed weight ([12]1000SW), was also more stimulated in wheat plants inoculated with *A. brasilense* Sp7 compared with inoculation with the NH strain or the noninoculated control plants (with and without algal extracts). The chlorophyll a ([13]CHA), b ([14]CHB), and the total contents of chlorophyll ([15]CHT) were slightly higher when algal extracts were added, as was the case after inoculation with Sp7 or NH (Table 3). Although the sugar content ([17]TS) was significantly reduced in inoculated plants (especially in the pot experiments), the proline content ([16]PRO) was only slightly affected by inoculation or addition of algal extracts (treatments 1, 2, 9, and 10).

Wheat growth was inhibited partially or completely in the presence of 160 and 200 mM NaCl in the pot and field experiments (treatment 3). The inoculation with *A. brasilense* Sp7 alone or together with algal extracts could not recover plant growth affected by the presence of salt (treatments 6 and 8) (Table 3). In contrast, the addition of *A. brasilense* NH alone and even more together with algal extracts (treatments 5 and 7) recovered wheat growth partially or completely (Table 3). As seen by comparing treatments 3 and 4 (salt inhibition without and with addition of *U. lactuca* extracts), algal extracts can recover to some extent plant growth affected by the presence of salt. The combination of inoculation with *A. brasilense* NH and addition of algal extracts under salt stress (160 or 200 mM) was more efficient than the separate application of *A. brasilense* NH or *U. lactuca* extracts (treatments 4, 5, and 7). Most plant parameters tested, like stem height, leaf length and width, stem and root dry weight, length and dry weight of spikes, seed numbers per spike, and total weight of seeds per spike all responded very similarly. In addition, the chlorophyll content of leaves presented the same response pattern. However, the 1000-seed weight revealed that if the plants could grow (they failed to grow at all at 200 mM NaCl with and without inoculation with Sp7; treatments 3 and 6), they reached about the same weight, with the inoculation with Sp7 giving the highest values (Table 3).

The contents of proline and total sugars were greatly elevated in the presence of salt stress. These contents

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Table 3 Evaluation of wheat growth under the various treatments

Code	Treatment	[1]Stem height (cm) ([1]SH)			[2]Leaf length (cm) ([2]LL)		
		Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)	Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)
T1	Control	60.20 ± 0.50^{Ea}	60.20 ± 0.50^{Ea}	66.10 ± 0.40^{Gb}	16.70 ± 0.71^{Ca}	16.70 ± 0.71^{Ea}	27.50 ± 0.20^{Fb}
T2	U	65.10 ± 2.43^{Ha}	65.10 ± 2.43^{Ga}	67.80 ± 0.61^{Hb}	25.10 ± 0.20^{Ga}	25.10 ± 0.20^{Ga}	28.30 ± 0.30^{Gb}
T3	NaCl	37.60 ± 0.50^{Ab}	$0.00 \pm 0.00A^{a}$	0.00 ± 0.00^{Aa}	15.70 ± 0.24^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T4	NaCl + U	46.90 ± 0.80^{Bc}	30.30 ± 4.83^{Ba}	41.50 ± 1.91^{Cb}	19.50 ± 0.34^{Dc}	12.30 ± 2.02^{Ca}	17.30 ± 0.80^{Cb}
T5	NaCl + NH	51.70 ± 1.73^{Dc}	38.60 ± 0.61^{Ca}	45.40 ± 2.60^{Db}	21.50 ± 0.72^{Fc}	$16.04 \pm 0.30^{\ Da}$	18.90 ± 1.10^{Db}
T6	NaCl + Sp7	38.01 ± 2.31^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	15.90 ± 1.30^{Bb}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T7	NaCl + NH + U	61.70 ± 0.53^{FGc}	45.90 ± 0.40^{Da}	47.20 ± 1.99^{Eb}	25.70 ± 0.30^{Ic}	19.04 ± 0.21^{Fa}	19.60 ± 1.10^{Eb}
T8	NaCl + Sp7 + U	48.70 ± 1.01^{Cc}	30.10 ± 5.72^{Ba}	38.10 ± 3.98^{Bb}	20.30 ± 0.41^{Ec}	12.30 ± 2.46^{Ba}	15.99 ± 1.90^{Bb}
T9	Sp7	62.10 ± 0.53^{Ga}	62.10 ± 0.53^{Fa}	69.80 ± 0.51^{Ib}	25.90 ± 0.41^{Ia}	25.90 ± 0.41^{Ia}	29.10 ± 0.23^{Ib}
T10	NH	61.20 ± 0.81^{Fa}	61.20 ± 0.81^{Fa}	64.20 ± 0.35^{Fb}	25.40 ± 0.42^{Ha}	25.40 ± 0.42^{Ha}	28.80 ± 0.20^{Hb}
Code	Treatment	[3]Leaf width (mm) ([3]LW)			[4]Stem dry weight (g) ([4]ADW)		
		Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)	Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)
T1	Control	6.22 ± 0.20^{Da}	6.22 ± 0.20^{Ea}	11.00 ± 0.00^{Eb}	1.98 ± 0.03^{Fa}	1.98 ± 0.03^{Fa}	2.21 ± 0.03^{Eb}
T2	U	7.50 ± 0.65^{Fa}	7.50 ± 0.65^{Ga}	11.00 ± 0.00^{Eb}	2.17 ± 0.10^{Ia}	2.17 ± 0.10^{Ia}	2.28 ± 0.02^{Fb}
T3	NaCl	5.00 ± 0.00^{Bb}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	0.30 ± 0.02^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T4	NaCl + U	5.98 ± 0.14^{Cc}	3.64 ± 0.70^{Ba}	4.93 ± 0.25^{Bb}	0.44 ± 0.01^{Cc}	0.20 ± 0.04^{Ca}	0.29 ± 0.02^{Bb}
T5	NaCl + NH	6.42 ± 0.54^{Ec}	5.00 ± 0.00^{Ca}	5.90 ± 0.40^{Cb}	1.27 ± 0.05^{Ec}	0.31 ± 0.01^{Da}	0.33 ± 0.02^{Cb}
T6	NaCl + Sp7	4.86 ± 0.45^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	0.32 ± 0.03^{Bb}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T7	NaCl + NH + U	8.00 ± 0.00^{Gc}	6.00 ± 0.00^{Da}	6.067 ± 0.25^{Db}	2.04 ± 0.03^{Hc}	0.41 ± 0.01^{Eb}	0.35 ± 0.02^{Da}
T8	NaCl + Sp7 + U	6.00 ± 0.00^{Cc}	3.50 ± 0.73^{Ba}	4.967 ± 0.50^{Bb}	0.47 ± 0.01^{Dc}	0.18 ± 0.05^{Ba}	0.28 ± 0.04^{Bb}
T9	Sp7	8.00 ± 0.00^{Ga}	8.00 ± 0.00^{Fa}	11.57 ± 0.50^{Fb}	2.04 ± 0.03^{Ha}	2.04 ± 0.03^{Ha}	2.30 ± 0.01^{Gb}
T10	NH	7.96 ± 0.20^{Ga}	7.96 ± 0.20^{Fa}	11.13 ± 0.35^{Eb}	2.02 ± 0.02^{Ga}	2.02 ± 0.02^{Ga}	2.28 ± 0.01^{Fb}
Code	Treatment	[5]Roots dry weight (g) ([5]RDW)			[6]Total spike length (hairs included) (cm) ([6]TSL)		
		Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)	Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)
T1	Control	0.49 ± 0.01^{Fa}	0.49 ± 0.01^{Fa}	0.64 ± 0.01^{Eb}	15.50 ± 0.12^{Ea}	15.50 ± 0.12^{Fa}	17.68 ± 0.28^{Fb}
T2	U	0.53 ± 0.02^{Ha}	0.53 ± 0.02^{Ga}	0.66 ± 0.01^{Fb}	17.14 ± 0.93^{Ha}	17.14 ± 0.93^{Fa}	18.45 ± 0.20^{Gb}
T3	NaCl	0.12 ± 0.01^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	10.14 ± 0.43^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T4	NaCl + U	0.19 ± 0.01^{Cc}	0.08 ± 0.01^{Ca}	0.10 ± 0.01^{Cb}	11.92 ± 0.40^{Bb}	8.57 ± 13.25^{Ca}	12.51 ± 0.90^{Cc}
T5	NaCl + NH	0.27 ± 0.02^{Ec}	0.14 ± 0.01^{Db}	$0.12 \pm 0.01^{\ Da}$	13.37 ± 0.51^{Dc}	$10.56 \pm 0.17^{\ Da}$	12.80 ± 0.63^{Db}
T6	NaCl + Sp7	0.14 ± 0.01^{Bb}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	10.17 ± 1.20^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}

Table 3 continued

Code	Treatment	[5]Roots dry weight (g) ([5]RDW)			[6]Total spike length (hairs included) (cm) ([6]TSL)		
		Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)	Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)
T7	NaCl + NH + U	0.52 ± 0.01^{Gc}	0.17 ± 0.02^{Eb}	0.12 ± 0.01^{Da}	15.83 ± 0.31^{Fc}	12.92 ± 0.25^{Ea}	13.41 ± 0.43^{Eb}
T8	NaCl + Sp7 + U	0.21 ± 0.10^{Db}	0.07 ± 0.02^{Ba}	0.09 ± 0.01^{Ba}	12.36 ± 0.41^{Cc}	6.62 ± 1.80^{Ba}	11.99 ± 0.80^{Bb}
T9	Sp7	0.62 ± 0.02^{Ja}	0.62 ± 0.02^{Ia}	0.69 ± 0.01^{Hb}	15.66 ± 0.33^{EFa}	15.66 ± 0.33^{Fa}	18.86 ± 0.30^{Hb}
T10	NH	0.61 ± 0.01^{Ia}	0.61 ± 0.01^{Ha}	0.68 ± 0.01^{Gb}	16.10 ± 0.22^{Ga}	16.10 ± 0.22^{Fa}	18.59 ± 0.24^{Gb}

Code	Treatment	[8]Spike length (hairs not incuded) (cm) ([8]SL)			[9]Spike dry weight (g) ([9]SDW)		
		Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)	Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)
T1	Control	6.44 ± 0.13^{Ea}	6.44 ± 0.13^{Da}	6.93 ± 0.06^{Fb}	2.70 ± 0.02^{Ea}	2.70 ± 0.02^{Fa}	2.91 ± 0.05^{Fb}
T2	U	6.73 ± 0.50^{Fa}	6.73 ± 0.50^{Da}	7.01 ± 0.06^{FGb}	2.90 ± 0.10^{Ga}	2.90 ± 0.10^{Ha}	2.97 ± 0.02^{Fb}
T3	NaCl	3.02 ± 0.10^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	1.60 ± 0.03^{Bb}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T4	NaCl + U	3.94 ± 0.40^{Bb}	3.71 ± 13.11^{Ca}	3.53 ± 0.20^{Ca}	2.10 ± 0.10^{Cc}	1.10 ± 0.30^{Ba}	1.90 ± 0.12^{Cb}
T5	NaCl + NH	4.40 ± 0.25^{Dc}	$3.03 \pm 0.10B^{Ca}$	3.70 ± 0.32^{Db}	2.40 ± 0.10^{Dc}	1.60 ± 0.02^{Da}	2.15 ± 0.20^{Db}
T6	NaCl + Sp7	3.03 ± 0.50^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	1.45 ± 0.20^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T7	NaCl + NH + U	6.50 ± 0.10^{Ec}	3.50 ± 0.12^{Ca}	4.06 ± 0.20^{Eb}	2.80 ± 0.05^{Fc}	2.12 ± 0.01^{Ea}	2.22 ± 0.10^{Eb}
T8	NaCl + Sp7 + U	4.05 ± 0.10^{Cc}	1.80 ± 0.45^{Ba}	3.31 ± 0.24^{Bb}	2.11 ± 0.05^{Cc}	1.15 ± 0.25^{Ca}	1.70 ± 0.30^{Bb}
T9	Sp7	6.50 ± 0.11^{Ea}	6.50 ± 0.11^{Da}	7.20 ± 0.20^{Hb}	$2.92 = 0.10^{Ha}$	2.92 ± 0.10^{Ha}	3.20 ± 0.02^{Gb}
T10	NH	6.52 ± 0.12^{Ea}	6.52 ± 0.12^{Da}	7.05 ± 0.11^{Gb}	2.80 ± 0.11^{Fa}	2.80 ± 0.11^{Ga}	3.13 ± 0.20^{Gb}

Code	Treatment	[10]Seeds number per spike ([10]SNS)			[11]Total seeds weight (g) ([11]SWS)		
		Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)	Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)
T1	Control	35.80 ± 0.70^{Fa}	35.80 ± 0.70^{Fa}	38.00 ± 0.00^{Fb}	2.30 ± 0.02^{Fa}	2.30 ± 0.02^{Fa}	2.45 ± 0.02^{Fb}
T2	U	37.96 ± 0.30^{Ga}	37.96 ± 0.30^{Ga}	38.00 ± 0.00^{Fa}	2.40 ± 0.05^{Ha}	2.40 ± 0.05^{Ha}	2.50 ± 0.01^{Fb}
T3	NaCl	19.92 ± 0.40^{Bb}	0.00 ± 0.00^{Aa}	0.00 ± 0.000^{Aa}	1.30 ± 0.03^{Bb}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T4	NaCl + U	25.50 ± 1.20^{Cc}	12.92 ± 2.50^{Ba}	22.70 ± 2.10^{Cb}	1.70 ± 0.09^{Cc}	0.80 ± 0.16^{Ba}	1.50 ± 0.10^{Cb}
T5	NaCl + NH	29.60 ± 0.93^{Ec}	18.00 ± 0.00^{Da}	25.60 ± 1.92^{Db}	1.95 ± 0.08^{Ec}	1.20 ± 0.03^{Da}	1.70 ± 0.22^{Db}
T6	NaCl + Sp7	17.60 ± 1.80^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	1.21 ± 0.15^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T7	NaCl + NH + U	36.00 ± 0.00^{Fa}	26.00 ± 0.00^{Eb}	26.80 ± 1.54^{Ec}	2.33 ± 0.02^{Gc}	1.70 ± 0.02^{Ea}	1.80 ± 0.09^{Eb}
T8	NaCl + Sp7 + U	25.96 ± 0.30^{Dc}	14.04 ± 2.93^{Ca}	19.93 ± 3.40^{Bb}	1.70 ± 0.05^{Dc}	0.90 ± 0.20^{Ca}	1.30 ± 0.23^{Bb}
T9	Sp7	36.00 ± 0.00^{Fa}	36.00 ± 0.00^{Fa}	38.00 ± 0.00^{Fb}	2.43 ± 0.04^{Ia}	2.43 ± 0.04^{Ia}	2.50 ± 0.01^{Fb}
T10	NH	36.00 ± 0.00^{Fa}	36.00 ± 0.00^{Fa}	38.00 ± 0.00^{Fb}	2.35 ± 0.10^{Ga}	2.35 ± 0.10^{Ga}	2.50 ± 0.02^{Fb}

Table 3 continued

Code	Treatment	[12]1000-seeds weight (g) ([12]1000SW)			[13]Chlorophyll a OD ([13]CHA)		
		Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)	Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)
T1	Control	64.22 ± 1.23^{Ba}	64.22 ± 1.23^{Da}	64.51 ± 0.70^{BCa}	2.50 ± 0.02^{Ea}	2.50 ± 0.02^{Fa}	2.80 ± 0.03^{Eb}
T2	U	63.12 ± 1.22^{Aa}	63.12 ± 1.22^{Ca}	65.20 ± 0.30^{EFb}	2.71 ± 0.05^{Ha}	2.71 ± 0.05^{Ha}	2.83 ± 0.03^{EFb}
T3	NaCl	64.70 ± 0.73^{Bb}	00.00 ± 00.00^{Aa}	00.00 ± 00.00^{Aa}	1.60 ± 0.02^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T4	NaCl + U	65.50 ± 1.10^{CDc}	60.30 ± 2.30^{Ba}	64.60 ± 2.20^{BCDb}	1.95 ± 0.03^{Bc}	1.30 ± 0.08^{Ca}	1.70 ± 0.12^{Bb}
T5	NaCl + NH	66.10 ± 1.54^{Dc}	64.90 ± 1.55^{EFb}	64.81 ± 7.02^{CDEa}	2.13 ± 0.20^{Dc}	1.60 ± 0.05^{Da}	1.90 ± 0.21^{Cb}
T6	NaCl + Sp7	68.80 ± 2.80^{Fb}	00.00 ± 00.00^{Aa}	00.00 ± 00.00^{Aa}	1.61 ± 0.06^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T7	NaCl + NH + U	64.74 ± 0.50^{Ba}	64.44 ± 0.80^{DEa}	65.84 ± 1.22^{Gb}	2.60 ± 0.01^{FGc}	1.91 ± 0.01^{Ea}	2.15 ± 0.21^{Db}
T8	NaCl + Sp7 + U	64.74 ± 1.97^{CDc}	62.90 ± 1.94^{Ca}	64.30 ± 1.11^{Bb}	2.02 ± 0.03^{Cc}	1.20 ± 0.21^{Ba}	1.70 ± 0.30^{Bb}
T9	Sp7	67.53 ± 0.99^{Eb}	67.53 ± 0.99^{Gb}	65.45 ± 0.34^{FGa}	2.60 ± 0.01^{Ga}	2.60 ± 0.01^{Ga}	2.90 ± 0.04^{Fb}
T10	NH	65.40 ± 1.99^{Cb}	65.40 ± 1.99^{Fb}	65.10 ± 0.50^{DEFa}	2.54 ± 0.03^{EFa}	2.54 ± 0.03^{FGa}	2.90 ± 0.02^{Fb}

Code	Treatment	[14]Chlorophyll b OD ([14]CHA)			[15]Total chlorophyll rate (mg/g FM) ([15]CHT)		
		Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)	Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)
T1	Control	2.30 ± 0.02^{Ea}	2.30 ± 0.02^{Fa}	2.60 ± 0.03^{Eb}	6.65 ± 0.10^{Ea}	6.65 ± 0.10^{Fa}	7.42 ± 0.10^{Eb}
T2	U	2.51 ± 0.06^{Ha}	2.51 ± 0.06^{Ha}	2.62 ± 0.03^{EFb}	7.24 ± 0.20^{Ha}	7.24 ± 0.20^{Ha}	7.55 ± 0.10^{EFb}
T3	NaCl	1.35 ± 0.03^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	3.99 ± 0.10^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T4	NaCl + U	1.74 ± 0.04^{Bc}	1.10 ± 0.08^{Ca}	1.50 ± 0.13^{Bb}	5.10 ± 0.10^{Bc}	3.25 ± 0.22^{Ca}	4.40 ± 0.40^{Bb}
T5	NaCl + NH	1.92 ± 0.20^{Dc}	1.20 ± 0.04^{Da}	1.70 ± 0.22^{Cb}	5.60 ± 0.50^{Dc}	4.11 ± 0.13^{Da}	4.90 ± 0.60^{Cb}
T6	NaCl + Sp7	1.41 ± 0.10^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	4.13 ± 0.20^{Ab}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T7	NaCl + NH + U	2.40 ± 0.02^{FGc}	1.71 ± 0.02^{Ea}	1.94 ± 0.21^{Db}	6.90 ± 0.04^{FGc}	4.99 ± 0.05^{Ea}	5.64 ± 0.60^{Db}
T8	NaCl + Sp7 + U	1.82 ± 0.02^{Cc}	0.98 ± 0.21^{Ba}	1.50 ± 0.30^{Bb}	5.30 ± 0.10^{Cc}	2.84 ± 0.70^{Ba}	4.23 ± 0.90^{Bb}
T9	Sp7	2.40 ± 0.02^{Ga}	2.40 ± 0.02^{Ga}	2.70 ± 0.04^{Fb}	6.93 ± 0.05^{Ga}	6.93 ± 0.05^{Ga}	7.80 ± 0.11^{Fb}
T10	NH	2.34 ± 0.03^{EFa}	2.34 ± 0.03^{FGa}	2.70 ± 0.04^{EFb}	6.80 ± 0.10^{EFa}	6.80 ± 0.10^{FGa}	7.75 ± 0.05^{EFb}

Code	Treatment	[16]Proline content (μg/g FM) ([16]PRO)			[17]Total sugars rate (μg/g FM)* ([17]TS)		
		Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)	Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)
T1	Control	2.10 ± 0.11^{Ab}	2.10 ± 0.11^{Ab}	1.90 ± 0.04^{Ba}	28.80 ± 0.98^{Ab}	28.80 ± 0.98^{Cb}	19.70 ± 0.33^{Ba}
T2	U	1.99 ± 0.05^{Ab}	1.99 ± 0.05^{Ab}	1.80 ± 0.06^{Ba}	25.98 ± 0.80^{Ab}	25.98 ± 0.80^{BCb}	18.92 ± 0.20^{Ba}
T3	NaCl	78.92 ± 1.30^{Db}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	252.90 ± 3.93^{Db}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
T4	NaCl + U	57.20 ± 1.93^{Ca}	92.99 ± 4.30^{Dc}	67.70 ± 0.6^{Eb}	183.20 ± 6.23^{Ca}	306.83 ± 10.74^{Fc}	214.40 ± 2.63^{Eb}
T5	NaCl + NH	49.20 ± 14.92^{Ba}	75.71 ± 2.10^{Cc}	57.50 ± 0.85^{Db}	157.45 ± 47.99^{Ba}	242.02 ± 6.22^{Ec}	183.85 ± 2.71^{Db}
T6	NaCl + Sp7	75.33 ± 2.90^{Db}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	240.90 ± 9.62^{Db}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}

Table 3 continued

Code	Treatment	[5]Roots dry weight (g) ([5]RDW)			[6]Total spike length (hairs included) (cm) ([6]TSL)		
		Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)	Pot expt. 1 (160 mM NaCl)	Pot expt. 2 (200 mM NaCl)	Field expt. (200 mM NaCl)
T7	NaCl + NH + U	2.02 ± 0.01^{Aa}	58.75 ± 0.75^{Bc}	51.50 ± 0.60^{Cb}	38.50 ± 57.11^{Aa}	187.80 ± 2.75^{Dc}	163.32 ± 1.23^{Cb}
T8	NaCl + Sp7 + U	52.91 ± 1.72^{BCa}	98.84 ± 11.65^{Ec}	67.93 ± 0.42^{Eb}	164.80 ± 7.40^{BCa}	317.31 ± 19.45^{Gc}	217.91 ± 2.04^{Fb}
T9	Sp7	2.01 ± 0.01^{Ab}	2.01 ± 0.01^{Ab}	1.74 ± 0.02^{Ba}	19.85 ± 0.33^{Ab}	19.85 ± 0.33^{Bb}	19.30 ± 0.55^{Ba}
T10	NH	2.03 ± 0.05^{Ab}	2.03 ± 0.05^{Ab}	1.80 ± 0.02^{Ba}	20.05 ± 0.15^{Ab}	20.05 ± 0.15^{Bb}	19.24 ± 0.41^{Ba}

FM fresh matter

The values are those of 17 parameters measured on adult plants. A–I and a–c express the statistical evaluation and compare the ten treatments (plots) (1–10) within each experiment (columns) and the three experiments within the same treatment (rows), respectively. Values ±SD accompanied by the same letter are not significantly different according to Fisher's least significant difference test ($P = 0.05$). The abbreviations after the parameters' names are their codes and, in left superscripts, their numbers in the parameters' list. [7] Beard hair length (cm) ([7]HL), not included in the table, is equal to [6] Total spike length (hairs included) (cm) minus [8] Spike length (hairs not included) (cm)

increased from treatment 7 to treatments 3 and 6 (7 < 5<8 < 4 < 3 and 6) and were proportional to stress intensity. The results of the durum wheat growth parameters under saline stress (160 or 200 mM NaCl) in pots and field experiments demonstrated that aqueous extracts of *U. lactuca* provide powerful osmoprotectants for the growth of durum wheat (*T. durum* var. waha), but their effect was less marked than inoculation with *A. brasilense* NH.

Correlation analyses clearly indicate relationships between the parameters measured in treatments 1–10 in the pot and field experiments. The principal component analysis (PCA) and their correlation are shown in Fig. 2a–d and electronic supplementary material Table 4.

Parameters like stem height, leaf height and width, stem and root dry weights, spike length and/or hairs, spike dry weight, number of seeds per spike, and total weight of seeds per spike clustered together, whereas the contents of proline and total sugar content were located at the opposite axis (Fig. 2). Therefore, these two parameters were inversely proportional to plant growth. In fact, when plants were grown under osmotic stress, proline and total sugar content increased, whereas other parameters were inhibited or decreased (Fig. 2a–d). In contrast, 1000-seed weight (1000SW) was not correlated with the aforementioned parameters, reflecting a very different regulatory mechanism controlling seed weight. The distribution of parameters of various treatments (1–10) revealed clearly that the measured values of treatments 1, 2, 7, 9, and 10 were more significantly clustered than those of treatments 3, 4, 5, 6, and 8 (Fig. 2a–d).

There was a strong correlation between all the parameters measured (including leaf height and leaf width) except the 1000-seed weight (Fig. 2a–d). The 1000-seed weight was superior in the pots with a high NaCl level. Because the plant would preserve its seed, the 1000-seed weight was proportional to osmotic stress (Fig. 2a–d). However, in pot experiment 2 (200 mM NaCl), 1000-seed weight was more correlated with factor 1 and less correlated with factor 2 (Fig. 2b) than in pot experiment 1 (160 mM NaCl) and in the field experiment (200 mM NaCl) (Fig. 2a, c). This suggests that salinity stress on leaf parameters was stronger in pot experiments than in the field experiment.

Principal component analysis revealed that the results obtained in the field experiment at 200 mM NaCl cluster into two groups: the first group contains treatments 1, 2, 9, and 10 in which no salt stress was applied, and the second group (treatments 4, 5, 7, and 8) in which there was salt stress (Fig. 3c). Treatments 3 (NaCl) and 6 (NaCl + Sp7) are missing because growth was completely abolished. Moreover, the similarity values obtained under various treatments in the field experiment were higher than those

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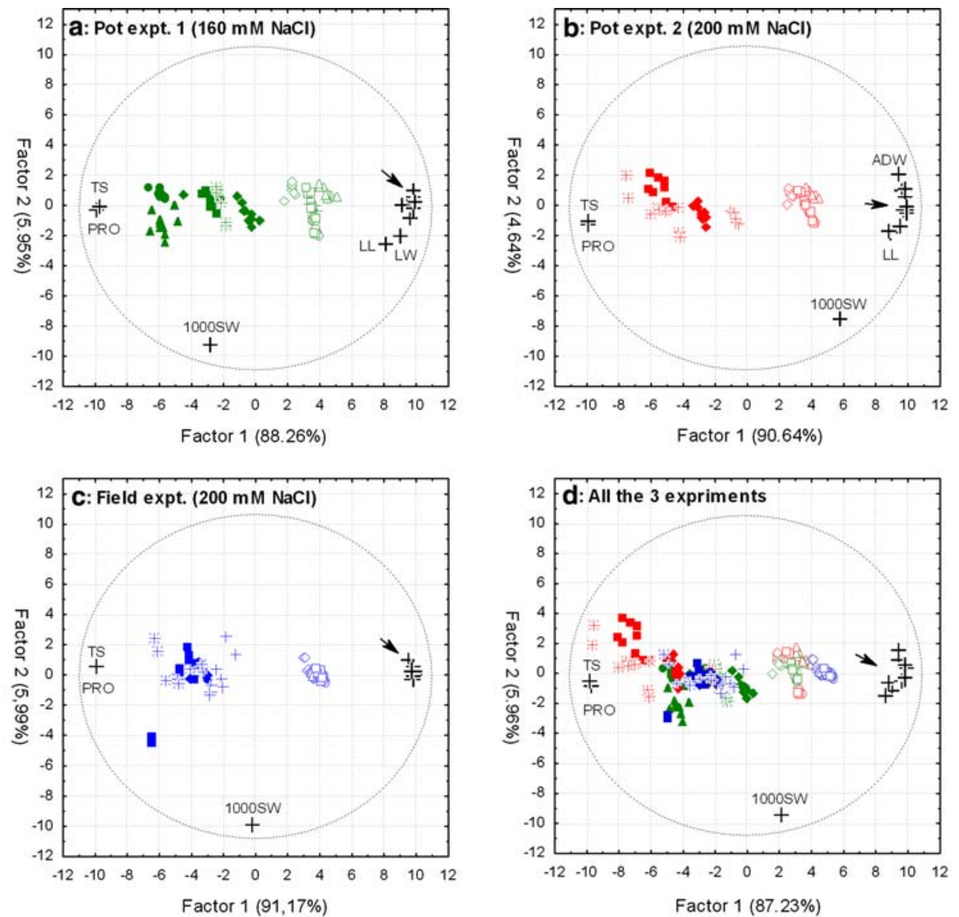

Experiments (1-3), Treatments (T1-T10) and variables (1-17) markers :

◇T1 △T2 ●T3 ■T4 ◆T5 ▲T6 +T7 ⁕T8 ○T9 □T10 ✚ Variables

The formulae of the 10 treatments: **T1:** Control (Wheat alone); **T2:** U; **T3:** NaCl; **T4:** NaCl + U; **T5:** NaCl + NH; **T6:** NaCl + Sp7; **T7:** NaCl + U + NH ; **T8:** NaCl + U + Sp7; **T9:** Sp7; **T10:** NH (See also Table 1 for detailed information); No plant growth under T3 and T6 at 200 mM NaCl in both Pot experiment 2 and field experiment (T3 and T6 not represented in b and c, and d at 200mM NaCl).

Correlation coefficient of the variables with the factors: R = variable coordinate/10);

The arrows point to the very closely gathered variables; they are in decreasing order, from top to bottom (factor 2), for **Pot expt. 1:** SNS, SL, TSL, CHT, CHB, CHA, SH, ADW, HL, SWS, SDW, RDW; for **Pot expt. 2:** ADW, CHT, SL, CHB, CHA, RDW, SNS, SWS, SH, TSL, SDW, LW, HL, LL; for **Field expt.:** HL, SNS, CHA, CHB, CHT, TSL, SDW, LL, SH, SWS, LW, ADW, RDW, SL.; for the 3expts.: ADW, RDW, CHB, CHA, SL, CHT, SNS, SH, TSL, SDW, SWS; **for all the 3 expts.:** ADW, RDW, SL, SNS, CHB, CHA, CHT, SH, SWS, SDW, TSL, LW, LL, HL.

Variables codes:
SH: [1]Stem height (cm); **LL:** [2]Leaf length (cm); **LW:** [3]Leaf width (mm); **ADW:** [4]Stem dry weight (g); **RDW:** [5]Roots dry weight (g); **TSL:** [6]Total spike length (hairs included) (cm); **HL:** [7]Hairs length (cm); **SL:** [8]Spike length (without hairs) (cm); **SDW:** [9]Spike dry weight (g); **SNS:** [10]Seeds number/ spike; **SWS:** [11]Total seed weight/ spike; **1000SW**: [12]1000-seed weight (g); **CHA:** [13]Chlorophyll a OD; **CHB:** [14]Chlorophyll b OD; **CHT:** [15]Total chlorophyll rate/fresh matter (mg/g); **PRO:** [16]Proline content/fresh matter (μg/g); **TS:** [17]Total sugars rate/ fresh matter (μg/g).
Statistic comparison of the PCA configurations
The Mean±SD positions for the different treatments and their statistic evaluation are given in Table 4

Fig. 2 Multifactorial comparison of the three experiments using PCA. The configuration in **d** (global PCA analysis of the three experiments) is the superposition of the partial configurations **a–c**. From the negative side of factor 1 to the positive side, the values for proline and total sugar contents decrease (strong negative correlation with factor 1) and increase for the remaining parameters except 1000-seed weight (strong positive correlation with factor 1). Similarly, the values for the 1000-seed weight decrease from the negative side of factor 2 to its positive side (strong negative correlation with factor 2) [see box under the figure and electronic supplementary material Table 4 for more information]

obtained in the pot experiments at 160 and 200 mM NaCl, respectively (Fig. 3a, b). The treatment with *U. lactuca* extracts under 200-mM-NaCl stress in pot experiment 2 was especially separate from the other value groups (Fig. 3b).

In Situ Localization of *A. brasilense* NH in Wheat Roots Using FISH Analysis and CLSM

In the monoxenic quartz sand culture conditions, wheat seedlings could grow only at 200 mM NaCl in the presence

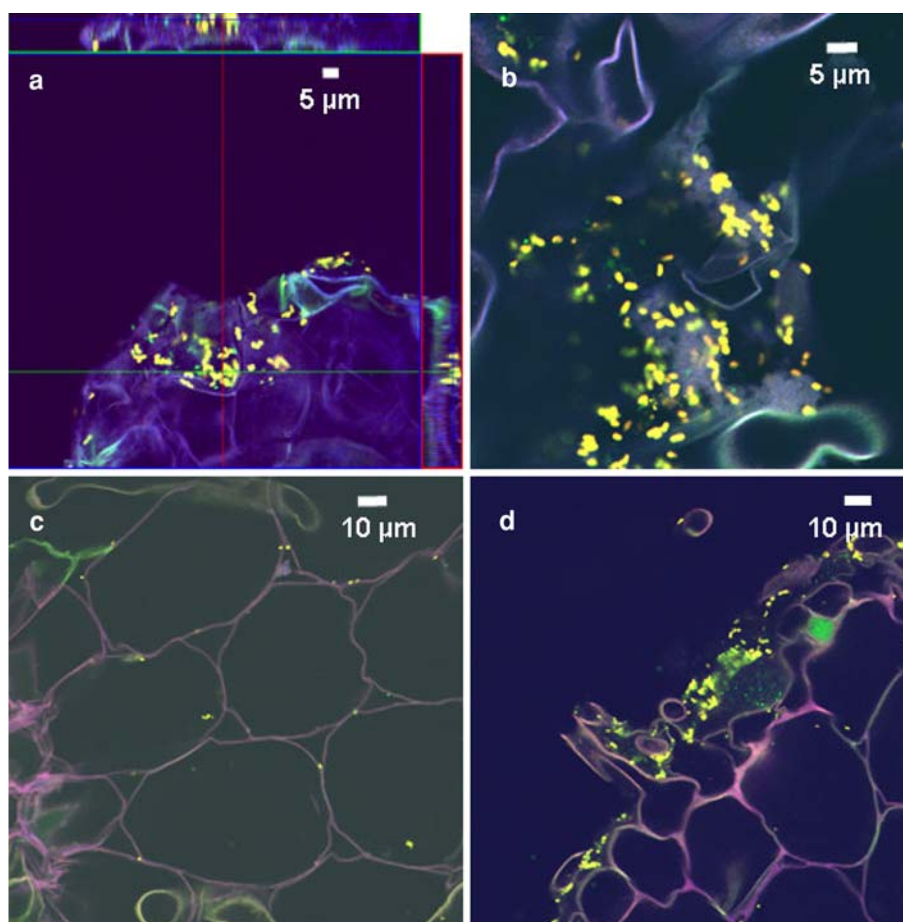

Fig. 3 Confocal laser scanning microscopic images of wheat roots inoculated with *A. brasilense* NH in axenic conditions after 4 weeks of growth under saline conditions (200 mM NaCl): The roots were fixed in 4 % PFA, FISH-analysis was performed using the probes EUB- 338-I, II, III-FLUOS and Abras-1420-Cy3. *A. brasilense* NH cells are stained brightly. **a** Endophytic colonization. Orthogonal optical sections of a three dimensional confocal image created from a z-stack of xy-scans. Vertical or horizontal optical cuts through the z-stack result in the side view images. **b** In situ detection of *A. brasilense* NH colonizing the root hair zone. **c**, **d** Radial slice from inoculated wheat roots. **c** Colonization of intercellular spaces. **d** Surface colonization

of *A. brasilense* NH. The in situ localization of *A. brasilense* NH using two oligonucleotide probes (EUB-338-I, II, III-FLUOS and Abras-1420-Cy3) concomitantly resulted in a clear localization and identification of single cells and small cell clusters on the root surface but also frequently within the roots (Fig. 3a–d). The predominant endophytic localization was the apoplastic space between the root epidermal cells.

ACC Deaminase Activity of *A. brasilense* NH

Using the growth assay on minimal medium plates with aminocyclopropane-1-carboxylate (ACC) as the sole nitrogen source, *A. brasilense* NH failed to grow in contrast to *B. phytofirmans* PSJN and other ACC-positive bacteria.

Discussion

The data presented demonstrate the exceptional ability of *A. brasilense* NH alone or in combination with aqueous extracts of the alga *Ulva lactuca* to restore and improve growth of durum wheat (*Triticum durum* var. waha) under saline stress conditions. Under nonsaline conditions, the aqueous extract of *U. lactuca* was also able to support wheat growth to some extent (Table 3). This can be explained by the nutritive value of the extract (see "Materials and Methods") and provides evidence that the composition and application rate of the algal extracts could improve the fertility of poor soils by providing it with P, N, and other essential growth elements (Alvey and others 2003; Nedzarek and Rakusa-Suszczewski 2004). In addition, both strains of *A. brasilense* (Sp7 and NH) could slightly stimulate wheat growth from germination to the harvest stage under nonsaline conditions (Table 2). The promotion of wheat growth by *A. brasilense* is probably due to two mechanisms: the production of phytohormones like indole acetic acid (Hartmann and others 1983) and the stimulation of β-glucuronidase in wheat roots (Kapulnik and others 1985; Kapulnik and others 1987; Vande Broek and others 1998). Moreover, *Azospirillum* is able to improve mineral uptake and to increase the nitrogen assimilation rate from the germination stage until the emergence of spikes (Ferreira and others 1987; Rodrigues and others 2000). On the other hand, the PGPR effect of *A. brasilense* Sp7 was noticed only in the absence of salt

stress because *A. brasilense* Sp7 is NaCl-sensitive. It had been demonstrated that salt stress severely altered the attachment of *A. brasilense* Cd, a very close relative to strain Sp7, to wheat roots by inhibiting both steps of adsorption and anchoring, which correlated with the impairment of the exopolysaccharide, glucane, and lipopolysaccharide contents (Jofré and others 1998). In addition, phytohormone biosynthesis and nitrogenase activity of *A. brasilense* Sp7 were inhibited under osmotic stress (Tripathi and others 2002). In contrast, *A. brasilense* NH reached its optimum of growth and indole acetic acid production at 200 mM NaCl (Nabti and others 2007).

Inoculation with *Azospirillum brasilense* NH improved germination at saline conditions greatly (Fig. 1), whereas application of algal extract had only a slight effect on the germination rate. Probably algal extracts provided osmolytes to the seed and thus retained water activity in the seeds (Nedzarek and Rakusa-Suszczewski 2004). Therefore, the combination of *A. brasilense* NH and algal extracts resulted in greatly improved germination compared with the control (treatments 1 and 7). The synergistic effect of these two treatments apparently improved the germination conditions to an extent equal to that in the absence of salt stress (Table 2). The results of partial inhibition of shoot and root development of durum wheat at 160 mM NaCl or total inhibition at 200 mM NaCl agreed with those obtained by Rengasamy (2002) and Rengasamy and others (2003). Osmotic stress reduced stem height, root length, and their dry weight. When environmental NaCl concentration increases, sodium uptake into roots increases as well, whereas N, P, K, and Mg uptake decreases and the intracellular ionic equilibrium is disturbed (Gunes and others 1996; Abdel-Ghaffar and others 1998). The PCA of the growth data of wheat in the pot experiments with 200 mM NaCl revealed that leaf length and width were not reduced to the same level as the other parameters (Fig. 2b). When wheat plants are cultivated under saline or water stress, the leaf responds initially by changes in volume and number of stomata to maintain sufficient water to restore the photosynthetic activity. The leaf should thus keep its surface intact to ensure good plant growth and development (Gallé and others 2002). The exposure of wheat seeds (*Triticum aestivum* L.) or barley (*Hordeum vulgare* L.) to 100 mM NaCl reduced the growth of their roots (length and dry weight) by 20%, whereas leaves remained intact (Termaat and others 1985). On the other hand, stem growth is very sensitive to osmotic stress (Rashid 1986). Similar results concerning stem, leaf, and root growth were obtained by Rashid (1986) and Iqbal and Mahmood (1992). The 1000-seed weight did not correlate with other growth parameters (PCA) (Fig. 2a–d). This could be because wheat seedlings affected by saline stress try to preserve their reserves (seed), whereas other parameters are altered (Evans 1993; Sayre and others 1997; Sharma and others 2005). When wheat plants were grown at high-salinity conditions, stem and root growth as well as the number of spikes and seeds were affected, but the 1000-seed weight remained more or less unchanged (Qureshi and others 1980; Francois and others 1986; Iqbal and Mahmood 1992).

The application of algal extract increased the chlorophyll content of wheat plants in the absence of saline stress (Table 3). *Ulva lactuca* extract probably provides plants with nutritive elements, thus enhancing photosynthesis. The same results were obtained with *A. brasilense* NH and Sp7 in the absence of salt. The effect of these two bacteria on the chlorophyll pigmentation could be due to improved mineral, nitrogen, and water uptake. The partial recovery of the chlorophyll content from markedly reduced chlorophyll levels at NaCl-stress conditions in the presence of *A. brasilense* NH or algal extracts and the complete recovery up to the control level with the combined application of *A. brasilense* NH and algal extracts were probably due to the concerted actions of several different beneficial effects. *A. brasilense* NH restored sufficient water and nitrogen content and the algal extracts provided the compatible solutes which reduced the inhibitory effect of increased salinity as well as several nutrients. The adaptation of plants to osmotic stress is associated with metabolic adjustments leading, for example, to the accumulation of organic compatible solutes such as sugars, polyols, betaines, and proline. Proline accumulation in plant tissues is an important physiological response to counterbalance saline stress. The greatly increased proline levels found in wheat plants during severe salt stress (Table 3) reflect this response. The inoculation of salt-stressed wheat with *A. brasilense* NH or the addition of algal extracts effectively reduced the proline content (Table 2). Because proline is a potent osmolyte accumulated under salt stress, this indicated reduced salt stress. The aqueous extract of *U. lactuca* provides a proline source, various betaines, and DMSP (Ghoul and others 1995) and other compatible solutes. Thus, under saline stress conditions, the wheat plants could directly use the compatible solutes available in the root environment because of the addition of algal extracts for osmotic adjustment. Salt-induced proline accumulation in the plants is reversed, providing again various amino acids necessary for growth and development (Brenda and others 2005). The contents of proline and chlorophyll measured in our study agree with those of other authors (Bengston and others 1978; Reddy and Veeranjaneyulu 1991), which suggests a possible connection between chlorophyll pigment biosynthesis pathways and the proline content. The accumulation of sugars is another important adaptive response of plants and other organisms to the lack of water and saline stress. Sugars play an important role during

germination of seeds under osmotic stress (Gill and others 2002). The accumulation of sugars in salinity stressed plants prevents structural and functional changes of membranes and destruction of soluble proteins. Soluble sugars (trehalose, mannitol, glucose, and fructose) are known for their osmoprotective effect in many species of wheat (Ashraf and others 1991; El-Haddad and O'Leary 1994). Our data of high total sugar contents at the most pronounced salt stress conditions (treatments 3 and 6, Table 3) coincides with this general response. Accordingly, the sugar content was reduced in treatments 4, 5, 7, and 8, when either algal extracts or *A. brasilense* NH or both were applied and osmotic stress was apparently reduced. The sugar content was found to respond to the experimental treatments exactly like proline content (Fig. 2). Salt-stressed *T. aestivum* L. plants responded also with increases in various sugar compounds (Kerepesi and Galiba 2000).

The improvement and the restoration of wheat growth under saline conditions by *A. brasilense* NH in part could be due to IAA production of this bacterium performing optimally at 200 mM NaCl (Nabti and others 2007). IAA is the most important phytohormone produced by *A. brasilense*. It was shown to be responsible for plant growth promotion from germination to the harvest stage and for morphology changes of roots, improved shoot growth, and improved yields when present at optimal concentrations (Baldani and Döbereiner 1980; Bashan and Levanony 1990; Barbieri and Galli 1993). The levels of phytohormones are crucial for the protection of the plant against various stresses. It was reported that salinity stress resulted in a progressive decline of IAA levels in wheat plants (Sakhabutdinova and others 2003). The ability of *A. brasilense* NH to produce IAA at salinity levels could complement this decrease in the IAA levels of the roots and thus restore the performance of the plant under salinity stress. In addition, *A. brasilense* was demonstrated to produce exopolysaccharides (EPS) under salt stress conditions (Konnova and others 1994). These EPS are released into the rhizosphere and form the EPS–cation–soil complex which is able to neutralize Na^+ ions in soil and prevent their uptake by the plant. This complex is also able to restore the water content in soil (Marshall 1975; Morel and others 1991; Bashan and others 2000). Another important mechanism of plant growth stimulation by rhizobacteria operates through the bacterial degradation by ACC deaminase of the precursor ACC of the phytohormone ethylene (Mayak and others 2004). Decreasing elevated ethylene levels in roots during salt stress leads to improved plant development. ACC deaminase is present in many PGPR (Glick and others 1998; Blaha and others 2006); however, our data do not support the presence of ACC deaminase activity in *A. brasilense* NH. Thus, ACC deaminase does not seem to be involved in the acquirement of halotolerance in this case.

It is most remarkable that FISH analysis using species-specific fluorescently labeled oligonucleotide probes combined with confocal laser scanning microscopy (Stoffels and others 2001) could demonstrate an abundant colonization of *A. brasilense* NH in the intercellular spaces of the wheat root cortex (Fig. 3). Endophytic bacteria were shown in several cases to exert the most interesting effects on plants, leading to efficient biological control, nitrogen fixation, and plant growth promotion (Rosenblueth and Martinez-Romero 2006; Schulz and others 2006). Within the species *Azospirillum brasilense*, a similar endophytic colonization is known for the strain Sp245, which also colonizes the root cortex of wheat plants (Schloter and Hartmann 1998; Rothballer and others 2003). Endophytic colonization of plants by microbes is possible only when the plant does not recognize the bacterial cells as an enemy and mobilizes defense reactions; on the other hand, the bacterium has the ability to enter and thrive in a plant successfully, exerting its beneficial effects with less competition with the abundant rhizosphere microflora. The observed apparently symbiotic interaction of the halotolerant and slightly halophilic strain NH with wheat roots may be regarded as a result of coevolution under saline conditions (Zilber-Rosenberg and Rosenberg 2008), which has prevailed over thousands of years in soils close to the Mediterranean coast of Algeria.

In conclusion the successful restoration of growth of durum wheat under salinity conditions after inoculation with *A. brasilense* NH with and without concomitant application of aqueous extracts of *U. lactuca*—a natural resource from the sea coast—provides the basis for a new approach of a successful formulation of a bacterial seed inoculum for the improvement of growth of durum wheat in saline soil.

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