World J Microbiol Biotechnol (2011) 27:197–205
DOI 10.1007/s11274-010-0444-1

ORIGINAL PAPER

Wheat (*Triticum aestivum* L.) growth enhancement by *Azospirillum* sp. under drought stress

M. H. Arzanesh · H. A. Alikhani · K. Khavazi · H. A. Rahimian · M. Miransari

Received: 28 March 2010 / Accepted: 6 May 2010 / Published online: 16 May 2010

Abstract Plant growth promoting rhizobacteria (PGPR) can enhance plant growth by alleviating soil stresses. Although previously investigated, some new interesting details are presented regarding the alleviating affects of *Azospirillum* sp. on wheat growth under drought stress in this research work. We hypothesized that the isolated strains of *Azospirillum* sp. may alleviate the adverse effects of drought stress on wheat (*Triticum aestivum* L.) growth. Three different strains of *Azospirillum lipoferum* (B1, B2 and B3) were used to inoculate wheat seedlings under drought. During the flowering stage the seedlings were subjected to three drought levels with five different time longevity, including control. Pots were water stressed at 80% (S0), 50% (S1) and 25% (S2) of field capacity moisture in a 25 day-period. Soil and plant water properties including water potential and water content, along with their effects on bacterial inoculum and wheat growth, were completely monitored during the experiment. While stress intensity significantly affected bacterial population and wheat growth, stress longevity only affected wheat water potential and water content. Compared to uninoculated treatments strain B3 (fixing and producing the highest amounts of N and auxin, respectively, with P solubilizing and ACC-deaminase activities) increased wheat yield at S1 and S2 by 43 and 109%, respectively. However, strain B2 (producing siderophore) was the most resistant strain under drought stress. The results of this experiment may elucidate the more efficient strains of *Azospirillum* sp. for wheat inoculation under drought stress and the mechanisms by which they alleviate the stress.

Keywords *Azospirillum* sp. · Drought stress · Seedling inoculation · Wheat (*Triticum aestivum* L.) growth

Abbreviations

ACC	1-aminocyclopropane-1-carboxylate
B1	*Azospirillum lipoferum* AZ1
B2	*A. lipoferum* AZ9
B3	*A. lipoferum* AZ45
S0, S1 and S2	Water stress treatments at 80, 50 and 25% of field capacity moisture, respectively
N	Nitrogen
P	Phosphorous
PGPR	Plant growth promoting Rhizobacteria

Introduction

Stress is a significant deviation from the optimum growth conditions. Under stress, plant demand for deficient resources increases resulting in plant growth reduction

M. H. Arzanesh
Department of Soil Science, Soil and Water Research Institute, Gorgan, Iran

H. A. Alikhani
Department of Soil Science, College of Agriculture, Tehran University, Tehran, Iran

K. Khavazi
Department of Soil Biology, Soil and Water Research Institute, Tehran, Iran

H. A. Rahimian
Department of Plant Pathology, College of Agriculture, Mazandaran University, Mazandaran, Iran

M. Miransari (✉)
Department of Soil Science, College of agricultural Sciences, Shahed University, 18151/159 Tehran, Iran
e-mail: Miransari1@Gmail.Com; Miransari@Shahed.Ac.Ir

(Hsiao 1973). Drought stress is common in many parts of the world and more than 50% of the globe is arid or semi arid or is subjected to some kind of drought stress. Drought stress can adversely affect plant growth and production. Plant response to drought stress, at cellular and molecular level, limits plant growth and yield (Pereyra et al. 2006).

Bacteria of the genus *Azospirillum* are among the most researched plant growth promoting rhizobacteria (PGPR) detected in the rhizosphere of many crop plants. Such bacteria are Gram-negative belonging to the α-subcalss of protobacteria group (Kloepper 2003; Cohen et al. 2008). They are able to produce plants hormones such as auxin, and proteins like polyamines, fix N, increase root growth and control pathogens. Such abilities collectively result in the enhanced growth of plants under stress (Ramos et al. 2002; Bhaskara Rao and Charyulu 2005; de-Bashan et al. 2005; Russo et al. 2008; Cassan et al. 2009).

Production of plant hormones by *Azospirillum brasilense* can increase root growth through enhancing nutrient uptake (Cohen et al. 2008; Russo et al. 2008; Pereyra et al. 2009). However, the mechanism by which they colonize the host roots is not yet elucidated (Ramos et al. 2002). The beneficial effects of *Azospirilum* on enhanced wheat yield and reduced chemical fertilization, including N fertilizer, are of important agricultural and environmental significance (Fischer et al. 2007; Spaepen et al. 2008). The competition with other soil microorganisms and the effects of soil chemical and physical properties may affect the *Azospirillum* potential when inoculating the host plant.

The genetically modified strains of *Azospirillum* are able to colonize plant roots and hence enter the host plant while competing with soil microorganisms (Jofre et al. 1998a, b; Rivarola et al. 1998; Barassi et al. 2006). Similar to plant species bacterial species are also subjected to drought stress and some of their physiological functioning are affected by the stress. Production of spore, cyst and some special types of cellular-like structures called C-form in the species of *Azospirillum* sp. are the mechanisms by which bacterial species can resist the unfavorable effects of stress. In addition, the effect of soil moisture on the activities of PGPR is a function of PGPR physical and physiological characters (Sadasivan and Neyra 1987; Bashan et al. 1991; Castellanos et al. 2000).

Bacterial mobility, their plant promoting and chemotaxic activities are affected by soil moisture. In addition, bacterial adsorption on the soil particles is related to the electrical properties of soil particles affected by soil moisture and pH. Decreased soil moisture or increased soil pH loosens bacterial attachment to the soil particles surfaces. Under adequate soil moisture and in the absence of the host plant, different PGPR species are adsorbed loosely on the surfaces of soil particles and hence can move to the roots of other plants (Oliveira et al. 2004).

Many researchers have indicated that *Azospirillum* spp. can alleviate the unfavorable effects of drought stress on plant growth (El-Komy et al. 2003; Arzanesh et al. 2009; Pereyra et al. 2006, 2009). In the one hand the association efficiency between *Azospirillum* and the host plant decreases under stresses such as water and nutrients deficiency. On the other hand, under intensified stress the ability of soil microorganisms to alleviate the stress (Miransari and Smith 2007, 2008; Miransari et al. 2007, 2008) may be enhanced. Such conditions are common in the arid and semi arid areas of the world.

In addition to decreasing *Azospirillum* population, increased salinity and drought significantly decreases the activity of nitrate reductase in plant and bacteria resulting in nitrate accumulation and hence decreased plant growth. As previously mentioned, *Azospirillum* is also able to produce plants hormones such as auxin and gibberellins in addition to the production of nitrate reductase even under stress, resulting in enhanced plant N and other nutrients uptake and hence plant growth (Hamdia and El-Komy 1998; Shinozaki and Yamaguchi-Shinozaki 2000; El-Samad et al. 2004; Barassi et al. 2006; Cohen et al. 2008; Hamdia et al. 2000; Spaepen et al. 2007, 2008).

Although there are previously documented results regarding the alleviating effects of *Azospirillum* on plant growth under drought, however the isolated strains and the method by which plants were subjected to drought stress in this experiment had not been previously tested. Additionally, soil and plant water characters including water potential and water content and their related effects on the bacterial population and wheat growth were completely monitored during the experiment. The objectives were to: (1) evaluate the effects of soil drought on *Azospirillum* population and wheat growth, and (2) test if inoculation of wheat plants with the isolated strains of *Azospirillum* sp. can alleviate drought stress on plant growth.

Materials and methods

Inoculum preparation

Three isolates of *Azospirillum* sp., including *Azospirillum lipoferum* AZ1 (B1), *A. lipoferum* AZ9 (B2), and *A. lipoferum* AZ45 (B3), (Table 1, isolated form the Iranian soils and supplied by DSMZ Company, Arzanesh et al. 2009) from the collection of Soil and Water Research Institute, Tehran, Iran, were grown on a RC (Rodríguez Cáceres 1982) medium, which is easily accessible. On the forth day of culturing using a platinum metal ring the bacterial cultures were collected and added to 15-ml Erlenmeyers containing New Fabian broth (NFB) liquid culture with

Table 1 The properties of the isolated *Azospirillum* sp

Isolates*	N-fixation (nano mole/h)	Auxin (mg/l)	P solubility (μg/ml)	Siderophore production	HCN production	Using ACC	Iranian region	Place of isolation
AZ45	39	35.40	8.59	−	1	0.012	Ardabail	Rhizoplane
AZ9	14.5	26.89	ND	+	1	0.018	Gorgan	Rhizoplane
AZ1	34.24	32.28	ND	−	1	0.025	Gorgan	Rhizopalne

ND Not determined

* *Azospirillum lipoferum*

NH_4Cl 0.1% (Dobereiner and Day 1975) and shaken at 120 rpm for 48 h at 30° C.

The number of *Azospirillum* sp. in the uninoculated (indigenous bacteria) and inoculated treatments were determined using the most probable (MPN) method. It is still a precise method for counting microbial population, in tubes containing a semi solid NFB medium (Dobereiner and Day 1975). It should be mentioned that the following isolates characters were also determined: (1) N-fixation (nano mole/h), (2) auxin production (in a medium with tryptophan after 120 h), (3) P solubility (using the quantitative method in a RC medium), (4) siderophore production (after 98 h on a Cas-Agar medium), (5) HCN production (on a RC medium with glycine), and (6) ACC utilization (Jalili et al. 2009; Abbas-Zadeh et al. 2010).

Seedling inoculation

Chamran wheat (*Triticum aestivum* L.) cultivar, from the Seed and Plant Improvement Institute, Karaj, Iran, was used for this experiment. Similar seeds with regard to their size and weight were selected and washed with distilled water three times. They were then soaked in alcohol 96% for 15 s. The extra alcohol on the seeds was removed and seeds were washed with sterilized distilled water. The seeds were then soaked in sodium hypochlorite 3% for 5 min and were washed with sterilized distilled water 5 times. The surface sterilized seeds were placed on Petri dishes with water and agar (10 g/l agar) and incubated at 20°C for 12 h and at 28°C for 60 h. Seedlings (with coleoptile length of 3–5 mm) were soaked in the bacterial solution with the population of 3.2×10^9/ml for 3 h.

Planting and treatments

The experiment was a factorial based on a completely randomized design including three drought intensities (subjected at five different time durations, including control) and four bacterial treatments in three replicates. Hence, 180 pots were used and three extra pots were used for time determination of drought stress. Twenty inoculated seedlings were planted at the 3-cm depth in each pot. Pots were irrigated according to their weight at 80% field capacity moisture. The minimum and maximum temperatures of 20 and 27°C, 16 h light at 15,000–20,000 mol photon m^{-2} S^{-1} using natural light and sodium and mercury lamps were the conditions in the greenhouse during the experiment.

At the three leaf-stages (V3), plants were thinned to 14 plants in each pot. Plants were subjected to the drought treatments starting at the flowering (20% flowering) stage, 50 days after planting (DAP) the seedlings. Drought treatments including control (soil moisture was kept at the 80% of field capacity moisture), moderate (50% of the field capacity moisture) and high drought (25% of the field capacity moisture) were used for the experiment. During the stress period pots were subjected to five intensities of water stress longevity including control; the first set was water stressed for only 6 days, however the second, third and fourth sets were water stressed for 12, 18 and 24 days, respectively, and then irrigation restored.

Experimental procedure

The experiment was conducted in the Research Greenhouse of the Agricultural Center of Golestan Province, Iran, for three months from 20th of September to 20th of December, 2007. The soil of one of the uncultivated research fields was used for the experiment. The amount of 1,800 kg soil from the 0 to 30 cm depth was collected for the experiment, sieved with 4-mm sieve, air dried and mixed with sand at the 8:2 ratio.

Soil physical and chemical properties were determined (Table 2). The soil properties including: (1) electrical conductivity (ECe) and pH of a saturated paste, (2) calcium carbonate percentage using hydrochloric acid and titration method, (3) organic C (Walkley and Black 1934) using sulfuric acid, (4) soil moisture at saturation, field capacity and permanent wilting point, and (5) soil texture (hydrometric method, Si.C.L) were determined. In addition, available P (Olsen 1954, using spectrometer), available potassium (using flame photometer) and total N (Kjeldahl method) were also measured. The amounts of micronutrients (mg/kg) including Fe, Zn, Cu (2.2) and Mn (2.4) were

Table 2 Soil physical and chemical properties

ECe (dS/m)	pH	$CaCO_3$ (%)	OC (%)	SP (%)	FC (%)	PWP (%)	Sand (%)	Silt (%)	Clay (%)
1	7.6	24	1.7	51	20	6	12	58	30

TN (%)	Ava.P (mg/l)	Ava.K (mg/l)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)
0.17	10.7	361	6.8	1.6	2.2	2.4

ECe electrical conductivity of saturated paste, *OC* organic carbon, *SP*, *FC* and *PWP* soil moisture at saturation, field capacity and permanent wilting point, *TN* total N, *Ava.P* available P, *Ava.K* available K

determined by diethylenetriaminepentaacetic acid (DTPA) method (Lindsay and Norvell 1978; Baker and Amachar 1982) using atomic absorption spectrometer (Model Perkin Elmer 3110).

Black pots with the diameter and height of 20 and 30 cm, (maximum volume of 10 kg soil), respectively were used for the experiment. According to the soil physical and chemical properties (soil testing) pots were ferti-irrigated (nutrients were dissolved in the water), when adjusting the soil moisture to the field capacity moisture, at 150 kg/ha urea, 175 kg/ha triple super phosphate and 200 kg/ha potassium sulfate. Phosphorous and K were fertilized at seeding and N was fertilized at three different stages including seeding (80 kg/ha), stemming (35 kg/ha) and grain filling (35 kg/ha) stages. Malathion (0.2%) was used twice during the growing season for controlling pests.

Soil and plant measurements

During the stress period leaf water potential and leaf water relative content in the flag leaf were determined. At 0, 6, 12, 18 and 24 days after the start of the stress, leaf water potential (four leaf) using the pressure chamber apparatus (Corvalis Oreg PMS Instrument Co.) and leaf water relative content (three leaf) and the related parameters including leaf turgor pressure and wet and dry weight were determined (Creus et al. 2004). At the same time soil matric potential was also determined using the time domain reflectometery method (TDR, Model TRIME-FM). In addition, at maturity grain and straw yield (g/pot), and yield components including the spikelet height and number, number of grains in each spikelet, 1,000-grain weight, ear height, and peduncle height, were also determined.

Statistical analyses

Analysis of variance (SAS Institute Inc 1988) for the main effects and their two- and three-way interactions was conducted (Steel and Torrie 1980). Means and their interactions were compared using the Least Significant Difference (LSD) test. In addition, coefficients of correlation for different growth parameters were determined.

Results

Drought stress, bacterial inoculation and bacterial population

The major factor increasing the population of *Azospirillum* spp. was seed inoculation with the bacteria under different conditions. The number of indigenous *Azospirillum* spp. in the control soil (uninoculated pots) ranged from 1.07 to 2.3×10^4/g dry soil at planting. After planting the seedlings and during the period before the onset of drought stress (50 DAP) the population of *Azospirillum* spp. in the same pots increased to 2.5×10^4, 4.57×10^4 and 2.4×10^4, at 80 (S0), 50 (S1) and 25% (S2) of field capacity, respectively. Although, drought stress at S1 and S2 significantly decreased the bacterial population at 75 and 90 DAP, bacterial inoculation significantly increased the number of bacteria in the soil at different intensities of stress relative to the control treatment (Table 3).

At S1 and 75 DAP, the number of *Azospirillum* in the pots inoculated with B1, B2 and B3, was 7.41, 8.32 and 7.24×10^4/g dry soil, respectively and at S2 the corresponding values were 3.80, 4.79 and 3.63×10^4/g dry soil, respectively. At S1 and S2 and relative to the control treatment the number of bacteria increased at 90 DAP, at all inoculated treatments, compared with 75 DAP treatment.

The response of different bacterial strains during the stress was different. The highest number of bacteria, at the end of the stress period (75 DAP), under inoculated and for S1 and S2 treatments was in the order of B2 > B1 > B3. At harvest and for S1 the related order was B3 > B2 > B1and for S2 it was similar to 75 DAP (Table 3). While, relative to S0 and 50 DAP, the bacterial population of B1 and B2 increased at S1, at 4.56 and 22.4%, respectively, the bacterial population of B2 and B3 at S2 decreased by 18.7 and 4.47%, respectively.

At the time that the stress was terminated the highest and adverse impact of drought on the bacterial population was observed, which was alleviated by the bacterial presence. At 75 DAP there was a significant decrease in the bacterial population; however, bacterial inoculation

Table 3 Number of *Azospirillum* sp. (per gram of dry soil) in soil inoculated with different strains of *Azospirillum* at different drought intensities and at different times after planting the seedlings

Drought level	Bacterial strain	At seeding	After 50 days	After 75 days	After 90 days
S0	B0	1.07×10^4	2.51×10^4	3.72×10^4	3.63×10^4
	B1	4.07×10^4	3.98×10^5	3.98×10^5	2.69×10^5
	B2	9.55×10^3	4.07×10^5	7.14×10^5	2.82×10^5
	B3	1.74×10^4	3.80×10^5	4.90×10^5	5.29×10^5
S1	B0	2.30×10^4	4.57×10^4	5.75×10^3	1.41×10^4
	B1	1.82×10^4	4.17×10^5	7.41×10^4	9.55×10^4
	B2	1.62×10^4	5.25×10^5	8.32×10^4	1.29×10^5
	B3	2.40×10^4	3.63×10^5	7.24×10^4	1.70×10^5
S2	B0	1.32×10^4	2.40×10^4	3.72×10^4	9.33×10^3
	B1	1.86×10^4	4.27×10^5	3.80×10^4	7.20×10^4
	B2	2.09×10^4	3.31×10^5	4.79×10^4	9.55×10^4
	B3	3.47×10^4	3.63×10^5	3.63×10^4	6.03×10^4

S0, S1 and S3: 80, 50 and 25% of field capacity moisture, respectively. B0: not inoculated, B1: inoculated with strain AZ45, B2: inoculated with strain AZ1, B3: inoculated with strain AZ9

Table 4 Matric potential (bar) values, at different drought intensities and at different times after planting seedlings

Drought intensity	After 50 days	After 56 days	After 62 days	After 68 days	After 74 days
S0	−5.00	−5.60	−6.03	−6.03	−8.01
S1	−8.28	−8.92	−9.94	−9.94	−10.41
S2	−10.72	−11.32	−11.82	−11.82	−13.74

partially increased the number of bacteria at different stress intensities, relative to the control treatment. At 90 DAP bacterial inoculation resulted in enhanced bacterial number for all inoculated treatments (Table 3). The trend of matric potential fluctuations in a 25-day period after the onset of drought stress is presented in Table 4. The alterations in soil matric potential during the first 18 days after the start of the drought stress was not much at different stress intensities, however, after 24 days, soil matric potential significantly decreased, relative to the previous 18 days period.

Grain yield and yield components as affected by different treatments

Although the most optimum plant performance was observed under non-stressed conditions, bacterial presence could notably alleviate the stress on wheat grain yield and components (Table 5). Treatment S0B3 produced the highest grain yield and related components; significantly different from the other treatments. The minimum amount of grain yield was related to treatment S2B0, significantly different from inoculated treatments.

Relative to uninoculated treatments strain B3 increased wheat yield at S1 and S2 by 43 and 109%, respectively. A similar pattern was also observed for 1,000-grain weight. However, for wheat straw the effects of bacterial strains were more pronounced at S1 relative to S2. Bacterial inoculation also significantly resulted in decreased leaf water potential and increased relative water content at S2 (Table 5).

Analysis of variance indicated that while drought stress, *Azospirillum* and their interaction significantly affected wheat growth parameters, the effects of stress longevity was significant only on leaf water potential and relative water content. In addition, the interaction effects of stress intensity and longevity, *Azospirillum* strains and stress longevity, and the interaction effects of all the experimental parameters on water leaf potential and relative water content were also significant (Table 6). High and significant correlation coefficients were determined among different wheat growth and physiological parameters (Table 7).

Azospirillum was also able to alleviate the drought stress on plant growth through adjusting plant water potential and content. With decreasing soil matric potential, leaf water potential increased. For S0, strain B3 had the highest effect on leaf water tension at different levels of soil matric potential, and for S1 and S2, strain B1. For S0 and S1 strain B3 influenced leaf relative water content most effectively

Table 5 Effects of *Azospirillum* strains on wheat growth and yield at different drought intensities

Treatment	Yield (g/pot)	Straw yield (g/pot) pot	weight of 1,000 grains (g)	Grain weight per ear (g)	Length of ear (cm)	Length of peduncle (cm)	Number of ears per pot	leaf water potential (bar)	Relative water content (%)
S0B0	16.40d	27.75bcd	36.29b	16.33 abcd	8.25b	21.04ab	16ab	−8.59a	90.26bc
S0B1	21.33b	31.82ab	36.43b	17.07abc	8.62b	21.81ab	17ab	−7.78a	92.01b
S0B2	23.07b	33.34a	35.88bcd	17.40abc	8.68b	22.21ab	17ab	−8.25a	91.87b
S0B3	27.02a	33.40a	44.28a	18.20a	9.57a	23.07a	18a	−7.39a	95.99a
S1B0	13.60e	24.05d	30.76bc	14.87cd	7.65c	12.80c	15ab	−12.81c	87.01d
S1B1	17.61cd	26.62cd	36.20bc	16.47abcd	8.55b	20.57ab	16ab	−11.48b	89.91bc
S1B2	16.38d	27.04bcd	35.28bcde	16.13abcd	8.62b	20.57ab	16ab	–11.80bc	87.68 cd
S1B3	19.42c	29.03abc	36.96b	17.87ab	8.62b	19.77b	18a	–11.91bc	92.28b
S2B0	8.21f	25.00cd	23.06f	14.27d	7.39c	9.03d	14b	−17.86f	80.64f
S2B1	13.39e	25.77cd	30.80de	15.40bcd	8.25b	19.98ab	15ab	−14.32d	86.9d
S2B2	14.47e	26.29cd	30.60e	15.87abcd	8.27b	20.56ab	16ab	−16.04e	83.73c
S2B3	17.19d	24.70cd	30.97cde	16.53abcd	8.28b	20.35ab	17ab	–12.34bc	82.07ef
LSD	1.85	4.80	5.26	2.75	0.58	3.098	3.21	1.23	2.61

S0, S1 and S3: 80, 50 and 25% of field capacity moisture, respectively. B0: not inoculated, B1: inoculated with strain AZ45, B2: inoculated with strain AZ1, B3: inoculated with strain AZ9. Means within the same columns and followed by different letters are significantly different at $P = 0.05$ using least significant difference (LSD) test

Table 6 Analysis of variance for different wheat parameters inoculated with *Azospirillum* sp. at different drought intensities imposed at different time duration

S.V.	df	Yield/pot	Weight of 1,000 grains	Number of ears per pot	Number of grains per ear	Length of ear	Length of peduncle	Straw weight/pot	Leaf water potential	Relative water content
S	2	1134.43**	1347.1**	45.12**	187.87**	8.08**	346.12**	631.44**	768.75**	1302.34**
B	3	548.91**	413.27**	42.61**	80.33**	9.03**	505.68**	113.68**	54.28**	160.43**
T	4	0.82	10.48	0.56	3.51	0.12	1.35	7.14	34.02**	93.81**
R	2	2.23	29.90	5.00	4.82	0.04	28.43**	31.06	0.063	4.34
S*B	6	21.87**	86.64	1.42	34.25**	0.94**	97.77**	31.16**	19.20**	61.35**
S*T	8	0.13	19.56	3.27	1.50	0.11	0.15	2.74	2.86**	10.68**
B*T	12	0.13	8.95	2.70	4.82	0.12	1.39	10.65	0.144	8.5**
S*B*T	24	0.11	7.78	2.60	2.24	0.14	0.98	11.93	1.93**	7.59**
Error	160	1.32	10.65	3.95	2.91	0.13	3.69	8.87	0.58	2.62
C.V.		6.61	9.61	12.14	10.43	4.26	9.95	10.67	6.51	1.83
R^2		0.96	0.80	0.44	0.74	0.785	0.87	0.69	0.96	0.93

S, drought stress; B, bacteria; T, stress duration; R, replicate; C.V., coefficient of variation; R^2, coefficient of determination

** Significant at $P = 0.01$

at different soil matric potential. However, similar to leaf water potential for S2, strain B1 also had the highest effect on leaf relative water content, at different soil matric potentials (Table 5).

Discussion

Affecting the cell wall leakage and water uptake is among the most important effects of salt and drought stress on plant growth (Table 7). As root exudates are necessary for the induction of bacterial genes in symbiotic or non-symbiotic associations, stress can indirectly affect the symbiosis. However, interestingly according to our results *Azospirillum* is able to survive and act even under drought stress. This indicates that the bacteria by itself and the amounts of root exudates, produced under stress are good enough for the onset and continuation of the bacterial activities.

PGPR including *Azospirillum* spp. affect plant growth through different activities including production of plant hormones such as IAA, produced by the expression of *ipdC*

Table 7 Correlation coefficients between different wheat parameters

	1	2	3	4	5	6	7	8	9
1. Grain yield	1								
2. Straw yield	0.62**	1							
3. Number of ears per pot	0.49**	0.27**	1						
4. Weight of 1,000 grain	0.75**	0.47**	0.38**	1					
5. Number of spikelet	0.71**	0.45**	0.60**	0.59**	1				
6. Peduncle length	0.69**	0.45**	0.40**	0.60**	0.53**	1			
7. Length of ear	0.76**	0.53**	0.41**	0.63**	0.65**	0.72**	1		
8. Leaf water potential	0.80**	0.54**	0.39**	0.69*	0.58**	0.59**	0.56**	1	
9. Relative water content	0.73**	0.53**	0.39**	0.71*	0.58**	0.49*	0.60**	0.74**	1

** Significant at $P = 0.01$

gene, N fixation and controlling pathogens (Spaepen et al. 2008; Jalili et al. 2009). Wheat root exudates such as nitrate and nitrite are among the root products, which are able to induce *Azospirillum* genes when interacting with the host plant (Pothier et al. 2007). Hence, under stresses, like drought, it is a matter of how the rhizosphere bacteria, specifically *Azospirillum*, can perceive plant biochemical products and how they can accordingly respond. This is determined by the strain specificity or in other words, the bacterial genome sequence (Setubal et al. 2009). Therefore, it can be stated that the strains that their stress genes become more activated and are more responsive to the host plant under drought stress are the more efficient ones. These biochemical products can act as signal molecules, enabling the bacteria to interact with the host plant similar to the N-fixing symbiotic bacteria (Miransari and Smith 2007, 2008). In addition, according to our results (Table 1) B3, which resulted in the highest wheat growth and yield under drought indicated the highest fixation and production of N and auxin, respectively, with P solubilizing and ACC deaminase activities. However, the most resistant strain was B2 producing siderophore. This indicates that such plant growth promoting abilities of bacteria determined the bacterial performance under different conditions including stress.

During the stress period the main reasons for the decreased bacterial populations are: (1) the time necessary for the adaptation of the inoculated *Azospirillum* sp. on the seedling roots and in the plant rhizosphere, and (2) production of inhibitory secondary metabolites including poly phenols and glutanins by the plants roots (Bais et al. 2006). Under common conditions the survival rate of *Azospirillum* sp. is higher than other rhizosphere PGPR. This is also verified by our results as even at the highest intensity of drought stress *Azospirillum* sp. survived (Konnova et al. 2001). When in association with the host plant, these bacteria are able to better tolerate soil stresses. Hence, under such conditions the bacterial population increases resulting in enhanced bacterial activities like N-fixation (Miller and Wood 1996).

After the stress period the bacterial populations were able to regenerate and their populations increased at harvest indicating that *Azospirillum* sp. are able to tolerate the drought stress and become active after the stress. This can be very interesting regarding these strains, because there are so many situations in which the agricultural soils are subjected to moisture fluctuations. With such kind of ability *Azospirillum* strains can alleviate the drought stress on plant growth and yield through stabilizing the plant growth conditions including plant water characters.

The results also indicate that different strains of *Azospirillum* sp. differ in their tolerance under drought stress. Strain B2 is the strain with the highest ability under drought stress. This can be of very important agricultural implications and finding the strains with the highest ability under different stresses would be very beneficial for using such bacteria as bioinoculants for different stress situations. Also illustrations of the mechanisms controlling such abilities in the bacterial strains can very much help to produce resistant strains for use under different stresses.

It should also be noted that plant inoculation is a favorable way of improving soil conditions for plant and bacterial growth. It is because according to our results increased bacterial population through inoculation increased the number of bacteria under stress relative to the control treatment. This is through the mutual and interactive behavior between the bacteria and the plant. Plant is able to provide some favorable conditions in the rhizosphere for microorganisms' activities, for example, by producing the root exudates. The bacteria, as previously mentioned, are particularly able to enhance the plant growth through alleviating soil stresses.

It is also very interesting that although drought stress decreased soil matric potential, however, differences in the potential fluctuations in the 18-day stress period was not significant and it was at 24 days after the onset of stress

that the difference became noticeable. This can be attributed to the soil texture, a silty clay loam, with 12, 58 and 30% of sand, silt, and clay particles and hence with a high water holding capacity. This is also the explanation for the effects of stress longevity on leaf water potential and water content and not on the parameters related to wheat growth. This indicates that the plant with the help of bacteria have developed some adaptation mechanisms under drought stress.

According to analyses of variance there is some kind of significant interactions between different parameters for example, between bacterial strains and stress intensities. This indicates that the different strains of bacteria perform differently under different intensities of stress and some strains may be able to perform more efficiently with increasing the stress intensity (Miransari et al. 2007, 2008; Daei et al. 2009; Miransari 2010a, b).

According to our results and relative to the control, bacterial strains decreased leaf water potential and increased leaf water relative content. This can be the explanation for the enhancing effects of *Azospirillum* strains on plant growth and yield. It is attributed to the production of plant hormones by bacteria, increasing plant growth through enhancing root growth and hence nutrient and water uptake (Casanovas et al. 2003; Creus et al. 2004, 2005; Cassan et al. 2009). In addition, different strains affected leaf water potential (which is the most effective parameter influeinccing crop yield, Table 7) differently indicating their various abilities under stress by producing different amounts of plant hormones (Table 1). It can be interesting to elucidate the different mechanisms producing such kind of differences.

Casanovas et al. (2003) performed a similar experiment with maize (*Zea mays* L.), subjecting plants to drought stress at flowering. They stated that drought at such a stage adversely affects leaf physiology and grain production. Inoculation of maize seeds with *Azospirillum brasilense* BR11005 resulted in the amelioration of such unfavorable effects by alleviating the stress of drought on plant growth. This is because *Azospirillum* spp. are able to enhance the formation of lateral roots (Creus et al. 2005) and produce different plant hormones including ABA (Cohen et al. 2008) and IAA.

According to our results, inoculation of wheat with *Azospirillum* spp. can alleviate drought stress on plant growth and yield through adjusting plant water characters. Drought longevity did not affect plant performance until 18 days after the onset of stress. Different bacterial strains performed differently under stress. The interactive effects between the experimental treatments indicate that the efficiency of some strains may enhance with increasing the intensity of stress. There are different mechanisms by which *Azospirillum* is able to alleviate drought stress on plant growth including the adjustment of plant water conditions, which is mostly due to the production of plant hormones affecting root growth and morphology. According to the results the most efficient strain under drought stress on wheat growth has been recognized, which may be used as a beneficial bioinoculant under such conditions.

References

Abbas-Zadeh P, Saleh-Rastin N, Asadi-Rahmani H, Khavazi K, Soltani A, Shoary-Nejati AR, Miransari M (2010) Plant growth promoting activities of fluorescent pseudomonads, isolated from the Iranian soils. Acta Physiol Plant 32:281–288

Arzanesh MH, Alikhani HA, Khavazi K, Rahimian HA, Miransari M (2009) In vitro growth of wheat (*Triticum aestivum* L.) seedlings, inoculated with *Azospirillum* sp., under drought stress. Int J Bot 5:244–249

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Ann Rev Plant Biol 57:233–266

Baker DE, Amachar MC (1982) Nickel, copper, zinc and cadmium. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, part 2, 2nd edn. American Society of Agronomy, Madison, pp 323–338

Barassi CA, Ayrault G, Creus CM, Sueldo RJ, Sobrero MT (2006) Seed inoculation with *Azospirillum* mitigates NaCl effects on lettuce. Sci Horticul 109:8–14

Bashan Y, Levanony H, Whitmoyer RE (1991) Root surface colonization of non-cereal crop plants by pleomorphic *Azospirillum brasilense* Cd. Microbiology 137:187–196

Bhaskara Rao KV, Charyulu PBBN (2005) Evaluation of effect of inoculation of *Azospirillum* on the yield of *Setaria italica* (L.). Afric J Biotechnol 4:989–995

Casanovas M, Barassi CA, Andrade FH, Sueldo RJ (2003) *Azospirillum*-inoculated maize plant responses to irrigation restraints imposed during flowering. Cereal Res Comm 31:395–402

Cassan F, Maiale S, Masciarelli O, Vidalc A, Lunaa V, Ruiz O (2009) Cadaverine production by *Azospirillum brasilense* and its possible role in plant growth promotion and osmotic stress mitigation. Europ J Soil Biol 45:12–19

Castellanos T, Ascencio F, Bashan Y (2000) Starvation-induced changes in the cell surface of *Azospirillum lipoferum*. FEMS Microbiol Ecol 33:1–9

Cohen AC, Bottini R, Piccoli PN (2008) *Azospirillum brasilense* Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in arabidopsis plants. Plant Grow Regul 54:97–103

Creus CM, Sueldo RJ, Barassi CA (2004) Water relations and yield in *Azospirillum*-inoculated wheat exposed to drought in field. Can J Bot 82:273–281

Creus CM, Graziano M, Casanovas EM, Pereyra MA, Simontacchi M, Puntarulo S, Barassi CA, Lamattina L (2005) Nitric oxide is involved in the *Azospirillum brasilense*-induced lateral root formation in tomato. Planta 221:297–303

Daei G, Ardekani M, Rejali F, Teimuri S, Miransari M (2009) Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. J Plant Physiol 166:617–625

de-Bashan LE, Antoun H, Bashan Y (2005) Cultivation factors and population size control the uptake of nitrogen by the microalgae *Chlorella vulgaris* when interacting with the microalgae growth-

promoting bacterium *Azospirillum brasilense*. FEMS Microbiol Ecol 54:197–203

Dobereiner J, Day J (1975) Associative symbiosis in tropical grasses characterization of microorganisms and dinitrogen fixing sites. In: International symposium on N2-fixation-interdisciplinary discussion. Washington State University Press, Pullman, p 518

El-Komy HM, Hamdia MA, El-Baki ABD (2003) Nitrate reductase in wheat plants grown under water stress and inoculated with *Azospirillum* spp. Biol Plant 46:281–287

El-Samad A, El-Komy HM, Shaddad MAK, Hetta AM (2004) Effect of molybdenum on nitrogenase and nitrate reductase activities of wheat inoculated with *Azospirillum brasilense* grown under drought stress. Gen Appl Plant Physiol 31:43–54

Fischer SE, Fischer SI, Magris S, Mori GB (2007) Isolation and characterization of bacteria from the rhizosphere of wheat. World J Microbiol Biotechnol 23:895–903

Hamdia MA, El-Komy MH (1998) Effect of salinity, gibberellic acid and *Azospirillum* inoculation on growth and nitrogen uptake of *Zea mays*. Biol Plant 40:109–120

Hamdia MA, El-Komy HM, Barakat N (2000) The role of foliar and potassium fertilization and/or p, *Azospirillium lipoforum* or *Bacillus polymexa* inoculation in nitrogen fixation and mineral nutrition of maize grown under salt stress. In: Xth international colloquium for the optimization of plant nutrition. April 8–13, Cairo Sheraton, Cairo-Egypt, p 193

Hsiao TC (1973) Plant responses to water stress. Ann Rev Plant Physiol 24:519–570

SAS Institute Inc (1988) SAS/STAT user's guide. Version 6, 4th edn. Statistical Analysis Instituite Inc, Cary North Carolina

Jalili F, Khavazi K, Pazira E, Nejati A, Asadi Rahmani H, Rasuli Sadaghiani H, Miransari M (2009) Isolation and characterization of ACC deaminase producing fluorescent pseudomonads to alleviate salinity stress on canola (*Brassica napus* L.) growth. J Plant Physiol 166:667–674

Jofre E, Fischer S, Rivarola V, Balengo H, Mori G (1998a) Saline stress affects the attachment of *Azospirillum brasilense* Cd to maize and wheat roots. Can J Microbiol 44:416–423

Jofre E, Fischer S, Rivarola V, Balengo H, Mori G (1998b) Differential gene expression in *Azospirillum brasilense* Cd under saline stress. Can J Microbiol 44:929–937

Kloepper JW (2003) A review of the mechanism for plant growth promotion by PGPR. In: Proceedings of 6th international PGPR workshop, Calcuta, India, pp 81–92

Konnova SA, Brykova S, Sachkova A, Egorenkova IV, Ignatov VV (2001) Protective role of the polysaccharide-containing capsular components of *Azospirillum brasilense*. Microbiology 70:436–440

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci Soc Am J 42:421–428

Miller KJ, Wood JM (1996) Osomoadaptation by rhizosphere bacteria. Ann Rev Microbiol 50:101–136

Miransari M (2010a) Arbuscular mycorrhiza and soil microbes. In: Thangadurai D, Busso C, Hijri M (eds) Mycorrizal biotechnology. CRP Press, USA, p 226

Miransari M (2010b) Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stresses. Plant Biol, in press

Miransari M, Smith DL (2007) Overcoming the stressful effects of salinity and acidity on soybean [*Glycine max* (L.) Merr.] nodulation and yields using signal molecule genistein under field conditions. J Plant Nut 30:1967–1992

Miransari M, Smith DL (2008) Using signal molecule genistein to alleviate the stress of suboptimal root zone temperature on soybean-*Bradyrhizobium* symbiosis under different soil textures. J Plant Interact 3:287–295

Miransari M, Bahrami HA, Rejali F, Malakouti MJ, Torabi H (2007) Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on corn (*Zea mays* L.) growth. Soil Biol Biochem 39:2014–2026

Miransari M, Bahrami HA, Rejali F, Malakouti MJ (2008) Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on wheat (*Triticum aestivum* L.) growth. Soil Biol Biochem 40:1197–1206

Oliveira ALM, El Canuto, Silva EE, Veronica MR, Baldani JI (2004) Survival of endophytic diazotrophic bacteria in soil under different moisture intensities. Braz J Microbiol 35:295–299

Olsen RS (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of Agriculture, No. 939

Pereyra MA, Zalazar CA, Barassia CA (2006) Root phospholipids in Azospirillum-inoculated wheat seedlings exposed to water stress. Plant Physiol Biochem 44:873–879

Pereyra MA, Ballesteros FM, Creus CM, Sueldo RJ, Barassi CA (2009) Seedlings growth promotion by *Azospirillum brasilense* under normal and drought conditions remains unaltered in Tebuconazole-treated wheat seeds. Europ J Soil Biol 45:20–27

Pothier JF, Wisniewski-Dye F, Weiss-Gayet M, Moënne-Loccoz Y, Prigent-Combaret C (2007) Promoter-trap identification of wheat seed extract-induced genes in the plant-growth-promoting rhizobacterium *Azospirillum brasilense* Sp245. Microbiology 153:3608–3622

Ramos HGO, Roncato-Maccari LDB, Souza EM, Soares-Ramos JRL, Hungria M, Pedrosa F (2002) Monitoring *Azospirillum*-wheat interactions using the *gfp* and *gusA* genes constitutively expressed from a new broad-host range vector. J Biotechnol 97:243–252

Rivarola V, Castro S, Mori G, Jofre E, Fabra A, Garnica R, Balegno H (1998) Response of *Azospirillum brasilense* Cd to sodium chloride stress. Antonie Van Leeuwenhoek 73:255–261

Rodríguez Cáceres E (1982) Improved medium for isolation of *Azospirillum* spp. Appl Environ Microbiol 44:990–991

Russo A, Vettori L, Felici C, Fiaschi G, Morini S, Toffanin A (2008) Enhanced micropropagation response and biocontrol effect of *Azospirillum brasilense* Sp245 on *Prunus cerasifera* L. clone Mr.S 2/5 plants. J Biotechnol 134:312–319

Sadasivan L, Neyra CA (1987) Cyst production and brown pigment formation in aging cultures of Azospirillum brasilense ATCC 29145. J Bacteriol 169:1670–1677

Setubal J et al (2009) Genome sequence of *Azotobacter vinelandii*, an obligate aerobe specialized to support diverse anaerobic metabolic processes. J Bacteriol 191:4534–4545

Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular response to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. Curr Opin Plant Biol 3:217–223

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448

Spaepen S, Dobbelaere S, Croonenborghs A, Vanderleyden J (2008) Effects of Azospirillum brasilense indole-3-acetic acid production on inoculated wheat plants. Plant Soil 312:1–23

Steel RGD, Torrie JH (1980) Principles and procedures of statistics: a biometrical approach, 2nd edn. McGraw-Hill Book Company, NY

Walkley A, Black IA (1934) An examination of degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci 37:29–37