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Plant Growth-Promoting Effects of Rhizobacteria on Abiotic Stressed Plants. *Azospirillum*-Grasses Model

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ABSTRACT

Plant growth-promoting rhizobacteria exert beneficial effects on plants when re-introduced by inoculation in a soil containing competitive micro flora. Amongst them *Azospirillum* is one of the most studied genera. Even though it colonizes different plant species in an ample variety of soils, it was first described in association with grass roots. In Argentina over 220,000 ha of wheat and corn were commercially inoculated with *Azospirillum* in 2008. In this review the management conditions leading to enhanced crop productivity are discussed. The beneficial effects of inoculation on abiotic stressed plants are also described. We present results showing how wheat and maize stress tolerance are enhanced due to *Azospirillum brasilense* Sp245 inoculation.

Keywords: inoculant, maize, plant-growth-promoting-rhizobacteria, water stress, wheat

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; **BNF**, biological nitrogen fixation; **IAA**, indole-3-acetic acid; **ISR**, induced systemic resistance; **IST**, induced systemic tolerance; **MPN**, most probable number; **NO**, nitric oxide; **PL**, phospholipid; **PC**, phosphatidylcholine; **PE**, phosphatidylethanolamine; **FA**, fatty acid; **PGPR**, plant growth-promoting rhizobacteria; **PEG**, polyethylene glycol

CONTENTS

IMPACT OF RHIZOBACTERIA ON AGRICULTURE

It is well recognized that the functionality of a terrestrial ecosystem depends on soil microbial activity (Doran 2002). Microorganisms in the rhizosphere react to the many metabolites released by plant roots. They also interact with plant roots by means of their own produced metabolites. In this scenario, signals derived from changes in the soil environment trigger selective root and shoot responses, as well as bacterial dynamic changes (Bais *et al*. 2006). Moreover, a sustainability analysis of crop production in terms of modern technological agriculture requires a detailed knowledge of the interrelationships between the microorganisms added to the system and those present in the soil. The result of these interactions would lead to a variety of positive, negative, and neutral effects on plants.

Among positive interactions, symbiotic ones between *Rhizobium* spp. and leguminous plants are traditionally the most studied (Gray and Smith 2005). Nevertheless, associative interactions like those produced by free-living rhizobacteria and roots are acquiring greater interest. Since several decades ago, these bacteria have been the focus of research because non-leguminous plants like rice, wheat and maize are the most important crops feeding the ever-growing human population on our planet (Bashan and Hartmann 2009).

About 2 to 5% of rhizobacteria exert a beneficial effect on plant growth when re-introduced by plant inoculation in a soil containing competitive microflora, and are so called plant growth-promoting rhizobacteria (PGPR) (Kloepper and Schroth 1978). They are free-living bacteria (Kloepper *et al*. 1989), and some of them invade the tissues of living plants and cause unapparent and asymptomatic infections (Sturz and Nowak 2000). PGPR may induce plant growth by direct or indirect modes of action (Beauchamp 1993; Glick 1995). Now, four mechanisms may be included into the direct mode. They are: 1) the production of phytohormones (Barbieri and Galli 1993; Dobbelaere *et al*. 1999) and stimulatory bacterial volatiles (Ryu *et al*. 2003); 2) the lowering of plant ethylene level (Glick *et al*. 1998); 3) the improvement of plant nutrient status by either making available those macro- and micronutrients from insoluble sources (Delvasto *et al*. 2006; Rodríguez *et al*. 2006; Hungría *et al*. 2010) or developing non-symbiotic nitrogen fixa-

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tion (Boddey *et al.* 2008); and 4) the stimulation of disease-resistance mechanisms (induced systemic resistance, ISR) (van Loon 2007). Indirect effects arose when PGPR act like biocontrol agents leading to reduce diseases (Compant *et al.* 2005), when they stimulate other beneficial symbioses, or when they protect the plant by degrading xenobiotics in inhibitory contaminated soils (Jacobsen 1997).

One important goal to improve agricultural performance and increase food production is to attain high yields, even at low soil fertility or without intensive fertilization. To achieve this goal, the use of PGPR in cropping systems seems to be a reliable practice. From extensive genetic, biochemical, and applied studies, *Azospirillum* is considered one of the best-studied PGPR, and it has been regarded as a general plant root colonizer (Vande Broek *et al.* 2000; Bashan *et al.* 2004). Numerous studies all over the world have shown that the application of *Azospirillum* improves crop productivity. *Azospirilla* inoculants are able to exert beneficial effects on plant growth and yield of many agronomic crops under a variety of environmental and soil conditions (Bashan and Levanony 1990; Sumner 1990; Fages 1994; Okon and Vanderleyden 1997). Even though there are many reports on successful experiments both in greenhouses and in the field, commercial application on large scale has lagged in the past decades (Bashan and Holguin 1997). This has been attributed largely to the unpredictability and inconsistency of field results (Okon and Labandera-Gonzalez 1994). Field and greenhouse experiments involving *Azospirillum* inoculation during the 1990's were carried out in many countries including Israel, France, Belgium, Argentina, Uruguay, Mexico and South Africa. These experiments' results were interesting reviewed by Okon and Labandera-Gonzalez (1994) and Dobbelaere *et al.* (2001). They concluded that inoculation with *Azospirillum* resulted in significant yield increases in the magnitude of 5–30% in about 60–70% of the experiments. The beneficial effects were mainly observed in lighter soils under intermediate levels of fertilizer (N, P and K) and water regimes (Okon and Labandera-Gonzalez 1994). They also stated that successful inoculation experiments appear to be those in which researchers had paid special attention to the optimal number of cells in the inoculants, using appropriate inoculation methodology, whereby an optimal number of cells remained viable and available to colonize roots (Okon and Labandera-Gonzalez 1994; Dobbelaere *et al.* 2001).

In the present decade, the use of commercial biological fertilizers is growing. Technically improved inoculants are now under development. They include formulations that can contain one or more bacterial strains or species enclosed in organic or synthetic, easy-to-use and economical carrier material. The inoculants' formulation has a crucial effect either on colonization or on plant growth promotion because the chosen formulation determines potential success (Bashan 1998). The progress made in this aspect probably may permit to increase the frequency of positive results. Apart from this type of so-called first-generation inoculants, which include only native bacteria (wild type cells), studies on genetically modified microorganisms are being conducted in order to evaluate the effects of improved second-generation inoculants (Dobbelaere *et al.* 2001; Baudoin *et al.* 2010).

Apart from the effect on yield increase, PGPR application might also increase nutrient uptake from soils, thus reducing the need for fertilizers and preventing water contamination with nitrates and phosphates accumulation in agricultural soils (Bashan *et al.* 2004). Some experiments have quantified the savings in chemical applications. For example, N savings were in the order or 20–50%, and provided superior results when organic fertilizers were incorporated (Bashan *et al.* 2004). Kennedy *et al.* (2004) proposed that inoculants used as biofertilisers, particularly N_2-fixing bacterial diazotrophs, could help to ensure that the nutrients supply contributing to optimized yield could be maintained. Results obtained from a three-year field research conducted to test whether microbial inoculants could be used to increase maize yield and to enhance nutrient uptake, were recently published (Adesemoye *et al.* 2008). They showed that inoculated plots removed higher amounts of N, P, or K from the soil, potentially reducing nutrient losses to the environment. A reduction in fertilizer application would lessen the effects of water contamination from fertilizers and lead to economical savings for farmers. This savings would increase the cost-benefit ratio, a crucial aspect for sustainable agriculture in many developing countries.

USE OF PGPR-BASED INOCULANTS IN ARGENTINA. EFFECTS AND CONDITIONS

The leading countries in applying *Azospirillum* at the field are Mexico, with an estimated 300,000 inoculated ha in 2007 followed by Argentina, where over 220,000 ha of wheat and corn were commercially inoculated with *Azospirillum* in 2008 (Bashan and Hartmann 2009). The yield potential of agricultural soils and the quality of crops might be enhanced by incorporating some selected PGPR into the soils, enriching the indigenous microbial population, and/or optimizing some soil functional processes (e.g., nutrient cycling, energy flow) (Caballero-Mellado *et al.* 1992). In Argentina, despite the growing tendency to adopt the inoculants' technologies and the fact that numerous bacterial-based inoculants developed for the main commercial crops are now available in the market (Maddoni *et al.* 2004), the fine tuning to achieve maximal efficiencies has not yet been attained (Diaz-Zorita *et al.* 2004). Pioneer studies in Entic Haplustols soils from the Pampas region of Argentina showed that wheat grain yield response to *Azospirillum brasilense* inoculation was dependent on differences in soil fertility and water availability (Rodríguez Cáceres *et al.* 1996). Even though several of these strains promoted greater tiller numbers, root dry matter and number of spikelets per plant, only INTA Az-8 or INTA Az-39 strains were able to promote a significant increase in grain yield (Puente *et al.* 2008). In this way, despite the lack of specificity in the interaction between the strain and the plant as in the case of a symbiotic relationship, a sort of potentiality amongst strains to develop different effects on crop production has been encountered. The INTA Az-39 strain was isolated from washed wheat roots originated in Marcos Juárez, Córdoba Province, Argentina (Rodríguez-Cáceres *et al.* 2008) and it is the native most studied strain. Inoculation with this strain resulted in a significant increase in wheat grain yield when evaluated in the semiarid region of Argentina (Rodríguez Cáceres *et al.* 1996). In a large study conducted during 2002–2006 growing seasons, the performance of a commercial inoculant based on INTA Az-39 strain was evaluated in 297 experimental field trials in the Pampas region of Argentina (Díaz-Zorita and Fernández Canigia 2008). Crop technology were adopted at each on-farm location according to the best locally recommended practices for achieving high wheat yields. At all the sites, wheat varieties sown were regionally adapted and recommended for high yielding environmental and crop management conditions. Nitrogen and P fertilization were applied when necessary according to recommendations based on chemical soil analysis and suggested protocols for each local site. Wheat grain yield from those 297 experimental sites varied in a range from 850 to 8050 kg ha^{-1} according to the management. The yield average increase was 260 kg ha^{-1}, equivalent to 8.0% of the mean wheat yield attained under the dry land farming conditions found in the region. Positive responses were determined in about 70% of the sites, depending mostly on the attainable yield and independently of fertilization and other crop and soil management practices. This is in agreement with the reported efficiency estimated from green house and field studies conducted in different parts of the world (Okon and Labandera Gonzalez 1994; Dobelaere *et al.* 2001). The interaction between inoculation and N and/or P-fertilization (biofertigation) showed that, as expected, fertilized wheat yield was enhanced with respect to that of the unfertilized crop. However, regardless of fertilization practice, inoculation significantly and positively affected yield, with mean yield responses of 259 and 260 kg ha^{-1} for unfertilized and fertilized wheat, respectively. In general, the grain yield response to inoculation was greater at sites under sub-humid moisture regime, or with deep soil profiles, than under semiarid environments or with shallow soils, respectively. Among the different soil types studied, only those soils subjected to both moisture regime limitation and shallows showed no significant grain production response to inoculation. This large assay allowed authors to conclude that in general, a greater response to inoculation is produced in the absence of major crop growth limitations, suggesting the complementary contribution of the *A. brasilense* treatment to more efficiently developing higher yielding wheat (Díaz-Zorita and Fernández Canigia 2008).

In a field trial conducted at the Estación Experimental Agropecuaria Balcarce of the Instituto Nacional de Tecnología Agropecuaria (INTA), we evaluated the response of *A. brasilense* Sp245-inoculated wheat to different water stress-applications. The soil was an Argiudoll typic (5.4% organic content, 11.5 ppm P, 8.85 ppm NO_3-N, pH 5.7, with an average deeper of 80 cm). All plots were fertilized with $(NH_4)_2HPO_4$ at sowing based on soil analysis. Inoculation treatment significantly raised grain yield when water stress was gradually imposed during a period ranging from 15 days prior to 15 days after the phenological state of anthesis. On the contrary, when the stress was applied from the onset of tillering, up to 15 days prior to anthesis, there were no inoculation positive effects on grain yield (Creus 1997).

García de Salamone *et al.* (1992) carried out a field experiment on three commercial maize genotypes with two *Azospirillum* strains isolated from Argentinean soils and strain Sp7 isolated from Brazilian soils. They found a significant statistical interaction between strain, maize genotype, and fertilization. Subsequent two field experiments on Argentinean and Brazilian maize genotypes were carried out. The inoculant consisted of a mixture of four *A. brasilense* strains isolated from surface-sterilized maize roots in Argentina and three *A. lipoferum* strains isolated from surface-sterilized maize or sorghum roots in Brazil. Significant inoculation effects on total N accumulation and on grain yields were consistently negative with one Argentinean genotype and positive with four Argentinean and two Brazilian genotypes, indicating an unknown interaction between the plant genotype and bacterial strain. Positive results were detected on enhanced grain yield, harvest index and N harvest index (García de Salamone and Döbereiner 1996). Biological nitrogen fixation (BNF) contribution to N harvest index differed between inoculant mixtures and maize genotypes. Inoculated maize grain yield and absorbed N varied between 7-43 and 5-46%, respectively, over non-inoculated controls values. Authors stated that their results strongly indicated that plant genotype-*Azospirillum* spp. strain interaction determines inoculation results on cereal crops. Inoculation's N contribution to the analyzed soils was in the order of 100 kg N ha^{-1}. Capelletti *et al.* (2004) carried out a field experiment on two pre-commercial *Azospirillum* inoculants with N and P fertilization. Grain yield, biomass production, and roots length and area were improved by inoculation between 6-17, 10-22, and 12-18%, respectively.

In addition, rain-fed rice response to inoculation with two locally isolated strains of *A. brasilense*, namely S1 and S2, was evaluated in a field trial conducted in Tucumán Province, Argentina (Pedraza *et al.* 2008). The authors found that each of both strains separately used as inoculum produced higher yield compared to non-inoculated controls, non-fertilized controls or inoculation combining both strains. Moreover, strain S2 produced the higher yield when it was applied together with urea fertilization. They also showed that even though urea-fertilization alone did not improve the total grain N content compared to the control, a significant improvement when applied together with different strains of *Azospirillum* was observed (Pedraza *et al.* 2008). Even though is well known that *Azospirillum* inoculated plants show a better nutrition (Bashan *et al.* 2004), there are yet scarce data that quantify the potentiality of this bacterium in Argentinean's agricultural systems.

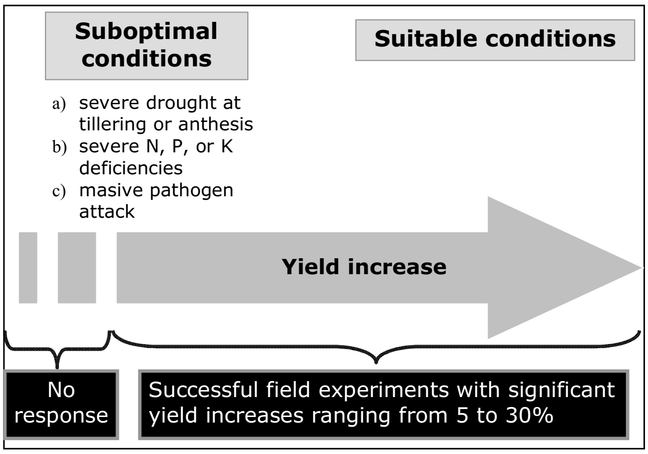

Fig. 1 Schematic representation of the general management conditions that lead to different increased yield percentages.

Altogether, these results point to a sort of threshold that limits the inoculants' effects. Under this threshold, which can be generalized as a severe stress condition, crop yield shows no increase. When the environmental and management conditions overcome that threshold and the availability of resources begins to improve, the boost generated by inoculation on the yield increases in a range that depends on the management condition. **Fig. 1** is a schematic representation of those conditions leading to different sized responses in yield.

RHIZOBACTERIA HELP PLANTS TOLERATE STRESS

In the biosphere, plants are often exposed to perturbations caused by the ambient where they live. It has been recognized that even though crop damage originated by pathogen attacks are the cause of substantial economic loses, the lack of water is the mayor threat against plant growth and crop production, even in humid areas (Boyer 1982). Coupled to the necessity for soil irrigation is the growing problem of field salinization worldwide. It was estimated that 10% of the world's cropland and as much as 27% of the irrigated land might be already affected by salinity (Shannon 1997). The gradual increase in salt content in irrigated soils is a growing menace for crop production (Kotb *et al.* 2000). By the other hand, abiotic factors can also directly influence PGPR activity and probably their effect on plant growth and the dynamics of root microbial communities.

Most studies on PGPR interactions with other soil microorganisms and with soil fauna have been focused on biocontrol or ISR against fungal, bacterial and viral diseases and against insect and nematode pests. The role of PGPR as elicitors of ISR in plants is well established (Kloepper *et al.* 2004; van Loon *et al.* 2004). Some rhizobacteria can reduce the susceptibility to diseases caused by plant pathogenic fungi, bacteria, viruses and nematodes. Although ISR-eliciting rhizobacteria can induce typical early defense-related responses in cell suspensions, in plants they do not necessarily activate defense-related gene expression. Instead, they appear to act through priming of effective resistance mechanisms, as reflected by earlier and stronger defense reactions once infection occurs (van Loon 2007). An interesting review on the ISR by PGPR in crop plants underlines the potential of *Pseudomonas* species for commercial exploitation and the possibility to develop mixed inoculants against various pathogens attacking the same crop at a time (Ramamoorthy *et al.* 2001).

In addition to the possible ISR mechanisms, a healthy plant would obviously be more able to cope with pathogens than a weakened one. Not to mention that a general response of plants to *Azospirillum* inoculation is a better water status than in non-inoculated ones, mainly expressed under water and/or saline stress conditions (Creus *et al.* 1997; Casanovas *et al.* 2002, 2003; Creus *et al.* 2004; Barassi *et al.* 2007). However, the effects that PGPR mediate on physiological and biochemical changes in plants under abiotic stress are less reported. Recently Yang *et al.* (2009) proposed the term 'induced systemic tolerance' (IST) for PGPR-induced physical and chemical changes in plants that result in enhanced tolerance to abiotic stress.

As a primary target, the root is the organ that shows the first stimulating bacterial effects. Indeed, the general effect upon inoculation with some PGPR is an increase in the development of the root system. One of the first observations regarding plant growth-promoting activity exerted by *Azospirillum* was on root size and morphology (Okon 1985). Based on these data, the possibility that a greater root system induced by rhizobacteria could lead to a greater exploration of soil resources, including water, was an interesting hypothesis established in the mid-1980's (Okon 1985). Further studies demonstrated that the positive effects of *Azospirillum* were mainly derived from morpho-physiological changes of the inoculated plant roots with an enhanced capacity for water and mineral uptake (Okon and Kapulnik 1986). In fact, many subsequent studies demonstrated that *Azospirillum* inoculation improved plant growth under stress conditions (Okon and Labandera-González 1994; Creus *et al.* 1998; Casanovas *et al.* 2002, 2003; Creus *et al.* 2004; Bacilio *et al.* 2004; Kokalis-Burelle *et al.* 2006; Nabti *et al.* 2010). After inoculation, the root displayed a significant increase in the number and length of root hairs, the rate of appearance and number of lateral roots, the diameter and length of lateral and adventitious roots, and the general root surface area (Kapulnik *et al.* 1985; Fallik *et al.* 1994; Dobbelaere *et al.* 1999; Creus *et al.* 2005). Besides, Levanony *et al.* (1989) reported an increase in cell division in the root tips of inoculated wheat. In addition, some reports showed that the inoculation of wheat or maize seedlings with *Azospirillum* cells resulted in an increased number of root hairs showing a Y-shaped deformation (Patrikin *et al.* 1983; Jain and Patriquin 1984; Kapulnik *et al.* 1985; Zamudio and Bastarrachea 1994).

Also wheat, maize and sorghum showed higher absorption of N, P and K depending on the *Azospirillum* strain inoculated (Kapulnik *et al.* 1985; Morgernsten and Okon 1987; Sarig *et al.* 1988; Hungría *et al.* 2010). Subjecting inoculated *Sorghum bicolor* plants to an osmotic stress in hydroponic systems diminished leaf senescence (Sarig *et al.* 1990).

On the other hand, the role of ethylene in the hormonal regulation of plant development has been well established. In spite of its effects on the development of some physiological processes like germination, a high level of ethylene concentration inhibits subsequent root elongation (Glick *et al.* 2007). During most phases of plant growth, ethylene production is minimal, but high levels may be synthesized as a response to environmental stresses, causing wilting and senescence (Abeles *et al.* 1992). Plants exposed to heavy metals and other stressors induce production of stress ethylene, which leads to premature plant senescence. In contrast, lowering ethylene levels mitigates harmful effects of many stressors (Reed *et al.* 2005). In addition, this molecule has been implicated in biotic stress, both as a virulence factor of fungal and bacterial pathogens and as a signaling compound in disease resistance (van Loon *et al.* 2006). One of the precursors of ethylene synthesis is 1-aminocyclopropane-1-carboxylic acid (ACC). ACC deaminase is the key enzyme, commonly found in many soil microorganisms, capable of degrading ACC. Thus, lowering ethylene levels in plants can be considered as having potential for promoting growth (Glick *et al.* 1998). Indeed, PGPR containing ACC deaminase can help plants to resist certain stresses and grow in under hostile environmental conditions (Reed *et al.* 2005).

Timmusk and Wagner (1999) studied changes in plant gene expression induced by inoculation with PGPR *Paenibacillus polymyxa* on *Arabidopsis thaliana.* They reported that inoculation with the PGPR enhanced drought tolerance. Subsequent challenge by either biotic or abiotic water stress indicated that inoculated plants were more resistant than control plants. Using RNA differential display technique, changes in gene expression were investigated. Several stress-responsive genes indicated that *P. polymyxa* induced mild biotic stress. Results suggest that genes and/or gene classes associated with plant defenses against abiotic and biotic stress may be co-regulated (Timmusk and Wagner 1999).

The protection that *Azospirillum* and other rhizobacteria could exert on plants suffering different kinds of biotic and abiotic stresses could be related to the phenomenon of cross-tolerance, by which a plant resistance to a stress result in the resistance to another form of stress (Genoud and Metraux 1999).

INOCULATION ON WHEAT AND MAIZE UNDER ABIOTIC STRESS. *AZOSPIRILLUM*–GRASSES MODEL

Azospirillum the best studied PGPR

The *Azospirillum* genus is included in the alpha subclass of Proteobacteria belonging to the IV rRNA superfamily (Xia *et al.* 1994). This group of free-living microorganisms encompasses 13 species, each one classified according to its particular biochemical and molecular characteristics: *A. lipoferum* and *A. brasilense* (Tarrand *et al.* 1978); *A. amazonense* (Magalhães *et al.* 1983); A. *halopraeferens* (Reinhold *et al.* 1987); *A. irakense* (Khammas *et al.* 1989); *A. largimobile* (Dekhil *et al.* 1997); *A. doebereinerae* (Eckert *et al.* 2001); *A. oryzae* (Xie and Yokota 2005); *A. melinis* (Peng *et al.* 2006), *A. canadensis* (Mehnaz *et al.* 2007a); *A. zeae* (Mehnaz *et al.* 2007b); *A. rugosum* (Young *et al.* 2008) and *A. picis* (Lin *et al.* 2009).

Azospirillum can fix atmospheric N_2 through the nitrogenase complex, when the availability of N compounds and oxygen tension are low (Döbereiner and Day 1976; Steenhoudt and Vanderleyden 2000). Even though this characteristic could be extremely valuable in agriculture, field studies including those in which isotopic dilution techniques were used, failed to demonstrate a significant BNF in *Azospirillum*-inoculated crops (Vande Broek *et al.* 2000). Even at the organism level, the growth promotion induced by the inoculation of axenic seedlings could not be ascribed to BNF (Bashan *et al.* 1989). Nevertheless, as was mentioned above, some experimental data demonstrated a significant BNF in maize crops (García de Salamone and Döbereiner 1996).

The production of phytohormones, namely auxins, cytokinins, and gibberellins, is the most commonly invoked mechanism of plant growth promotion exerted by PGPR. Among them, auxins are thought to play the major role. Even though it was suggested more than 60 years ago that rhizobacteria could produce auxins (Roberts and Roberts 1939), it was only in the 1970's that this assumption was proved (Brown 1972; Barea and Brown 1974; Tien *et al.* 1979). Nowadays it is well known that *Azospirillum* can synthesize indole-3-acetic acid (IAA) by at least three different pathways. Controlled experiments *in vitro* showed that IAA content increased in roots and shoots of *A. brasilense* FT326-inoculated tomato (Ribaudo *et al.* 2006). To evaluate the involvement of bacterial IAA in the promotion of root development, several investigations were conducted with mutant strains altered in IAA production. Some experiments showed a reduced ability to promote root system development with different kinds of auxin mutants (Barbieri and Galli 1993; Kundu *et al.* 1997; Dobbelaere *et al.* 1999). However, there are no reports showing to what extent IAA is produced in the rhizosphere by *Azospirillum* (Lambrecht *et al.* 2000; Steenhoudt and Vanderleyden 2000). Moreover, the role of chemical signals in mediating rhizospheric interactions is beginning to be understood (Bais *et al.* 2006). Among the signals that are potentially involved in root growth, N species as nitrate and NO (nitric oxide) are implicated in root growth and development (Lamattina *et al.* 2003). Creus *et al.* (2005) have reported the NO production by *Azospirillum* growing under aerobic conditions. The analogies found between the experimental data concerning *Azospirillum* stimulation of plant root development and the capability of NO to act as a non-traditional plant growth regulator (Beligni and Lamattina 2001) promoting adventitious root formation, lateral root development, and root hair formation, led us to explore whether *Azospirillum* ability to promote root growth and modify root architecture relies on NO. Recently we demonstrated that when NO is removed from the interaction between root and *Azospirillum*, both lateral and adventitious root formation were inhibited and attained to the non-inoculated values, evidencing that NO is strongly involved in the *Azospirillum*-induced root branching (Molina-Favero *et al.* 2007, 2008).

Other special characteristics present in *Azospirillum* make it a valuable PGPR. Some of them are briefly summarised below.

The bacterium is established mainly on the root surface but some strains of *A. lipoferum* and *A. brasilense* are endophytes colonizing the apoplast and intercellular spaces of the root. This ability could mean a lower vulnerability to harsh conditions imposed by the environment (Sturz and Nowak 2000). Indeed, it has been published that *A. brasilense* and *A. halopraeferans* can withstand growth in seawater for more than 30 days (Puente *et al.* 1999). *Azospirillum brasilense* Sp245 (EMBRAPA-Rio de Janeiro, Brazil) initially isolated from sterilized wheat roots (Baldani *et al.* 1983) shows a high colonizing potential, and can be found in different parts of the root, including the inner root tissue, forming micro-colonies in intercellular spaces (Bashan *et al.* 2004). We evaluated the growth of *A. brasilense* Sp245 cells in N-free semi-solid media supplemented with either 160 mM NaCl, 320 mM NaCl, 20% polyethylene glycol (PEG) 6000, or 30% PEG 6000. Bacterial produced typical white growth pellicles under the surface showing in addition that the N-fixing capability is functional under these circumstances (Creus *et al.* 1997). Our findings also showed that *A. brasilense* Sp245 could tolerate up to 20% PEG 8000 in a chemically defined liquid medium (OAB broth) without appreciable decline in cells number (data not published). *Azospirillum* spp. can accumulate compatible solutes, mainly trehalose, proline, and glutamate depending on N source (Madkour *et al.* 1990) to allow adaptation to fluctuations in soil salinity or osmolarity (Bashan *et al.* 1997).

Azospirillum spp. is not considered a classic biocontrol agent of soil-borne plant pathogens. However, there have been reports on moderate capabilities of *A. brasilense* in biocontrolling some different pathogenic agents (Bakanchikova *et al.* 1993; Sudhakar *et al.* 2000; Bashan and de-Bashan 2002). These antibacterial activities of *Azospirillum* could be related to its already known ability to produce bacteriocins (Oliveira and Drozdowicz 1987) and siderophores (Tapia-Hernández *et al.* 1990; Shah *et al.* 1992) or phenylacetic acid, an auxin-like molecule with antimicrobial activity (Somers *et al.* 2005).

As the most researched associative bacterium, *Azospirillum* has become a cornerstone of rhizosphere research unrelated to its direct agricultural application. *Azospirillum* is an excellent model for studies of plant-associative bacteria in general. In nature, a broad host range may help bacteria survive better. Reports about isolating *Azospirillum* from graminaceous plants were very common in the past, and so claims of *Azospirillum* specificity for certain cereal species were frequently published (Bashan and Levanony 1990). They stated that *Azospirillum* mainly enhanced growth of cereal plants. The data published in recent years, however, show otherwise. They show that the bacterium is a natural inhabitant of many non-graminaceous plants. *Azospirillum* strains had no preference for crop plants or weeds, or for annual or perennial plants, and can be successfully applied to plants that have no previous history of *Azospirillum* in their roots. It appears that *Azospirillum* is a universal bacterium found almost everywhere, a general root colonizer and is not a plant specific bacterium (Bashan and Holguin 1997). The full host range of *Azospirillum* has not yet been defined. We propose the grasses–*Azospirillum* interaction model, in an attempt to generalize the plant promotion response under stress conditions, after twenty years of studies on the effects of *Azospirillum brasilense* Sp245 inoculation in wheat and corn.

Azospirillum brasilense Sp245 root colonization

A successful *Azospirillum* colonization either of the rhizosphere, the surface and/or the interior of the root is determinant to enhance plant growth and crop yield (Dobbelaere *et al.* 1999). Several authors have reported that the expression and the magnitude of the plant-bacterium interaction would depend on the number of cells that colonize the root, and this number differs according to the plant species. The bacterial establishment is affected by various edaphic, abiotic and biotic factors, which also have direct influence on the plant growth and the functioning of the root (Vande Broek and Vanderleyden 1995). The inoculation method must ensure the liberation of the inocula in the field in a timely and precise way, allowing the bacterial cells to remain viable and to keep their ability to colonize the roots in an adequate number (Okon and Labandera-González 1994). This number varies according to plant species, being the recommended one 10^5-10^6 bacteria per plant 2-3 weeks after sowing in wheat (Kapulnik *et al.* 1985) and 10^7 in the case of maize (Arsac *et al.* 1990).

In our lab, we developed a simple method to obtain inoculated seeds containing high number of viable *Azospirillum* cells avoiding the use of external carriers or adhesives (Creus *et al.* 1996). Wheat seeds inoculated during imbibition and dried to 14% water content retained 3.7×10^6 viable cells g^{-1} dry weight up to 27 days after bacterial treatment (Creus *et al.* 1996).

High cell concentrations in the roots might cancel out the growth promoting effect (Dobbelaere *et al.* 1999). A significant decrease in the size of the elongation zone of the roots was reported at higher inoculum levels (10^8 bacterial cells ml^{-1}) (Dobbelaere *et al.* 1999). Our results showed that since *A. brasilense* Sp245could have been multiplying inside the plant roots, root-colonization should be taken into account instead of inoculum concentration (Pereyra *et al.* 2007). Root colonization, expressed as most probable number of diazotrophic bacteria (MPN) should not exceed 2.5×10^8 *A. brasilense* Sp245 cells in *T. durum* cv. 'Buck Topacio' seedlings, since when 3.3×10^8 cells were colonizing seedling roots, no growth promoting effects were detected (Pereyra *et al.* 2007). We concluded that to diminish the risks of overloading the roots and loosing the beneficial growth promoting effects exerted by *A. brasilense* Sp245 it would be advisable to use an inoculum containing less than 5×10^5 bacterial cells per seedling in cv. 'Buck Topacio' of durum wheat (Pereyra *et al.* 2007).

Not only high but also low cell concentrations in the roots might cancel out the growth promoting effect (Dobbelaere *et al.* 1999). Mild osmotic stress (-0.54 MPa) altered *A. brasilense* Sp245-wheat seedlings interactions, diminishing total root colonization in one order (10^7 to 10^6 cells g DW $roots^{-1}$) (Pereyra *et al.* 2006). In spite of this, *Azospirillum* inoculation promoted significant increases in coleoptile length and projected area, and in root length (Pereyra *et al.* 2006).

Maize seeds were inoculated with 10^7 *A. brasilense* Sp245 cells $seed^{-1}$, air-dried to 14% humidity and dry stored at 15-20°C in the dark up to 25 days. After 0, 5, 15 and 25 days of storage seed were germinated and seedlings grown for 5 days. MPN in roots of seedlings from inoculated seeds decayed from 10^8 cells g^{-1} at 0 day to 10^5 cells g^{-1} at day 25. However the growth promoting effect on root and coleoptile was maintained (Casanovas *et al.* 2000).

Another important aspect to achieve proper root colonization, in view of the high input of agrochemicals in contemporary crop production, is the compatibility between *Azospirillum* inoculants with common pesticides. Our results in laboratory tests showed that *A. brasilense* Sp245 cells growth was not hampered by Tebuconazole, a fungicide widely used to prevent the most common fungal diseases in wheat seeds. No effects were detected during exponential growth up to 14 h, when bacteria reached the metabolic optimum conditions to interact with and colonize seedling roots (Pereyra *et al.* 2009). Tebuconazole also did not affect *A. brasilense* Sp245 wheat root colonization when present in the hydroponic media in either normal or water stress conditions (Pereyra *et al.* 2009). Opposite results were found by Puente *et al.* (2008) in Tebuconazole treated *A. brasilense* Az39 INTA inoculated wheat seeds which presented a lesser number of cells than non treated controls.

Wheat and maize seedling growth promotion

The lack of water is one of the main constraints to plant growth and crop yield. Many reports indicate that the best performance of *Azospirillum* in the field is associated with non-optimal conditions for plant growth (Bashan *et al.* 2004), unless the conditions are bellow the threshold of severe stress (see above in this review).

Stress conditions after planting affect both germination and initial growth, reducing the obtained plant density and consequently the final yield per unit area. In wheat, successful seedling establishment is highly dependent on proper coleoptile development. This specialized tissue could have an important role not only in seedling survival when drought strikes shortly after seeding but also in much later stages as physiological maturity, influencing grain yield at harvest (Gan *et al.* 1992). Seedling survival to water stress in the days following germination is one of the major limitations to the establishment of species in many habitats (Lafond and Fowler 1989). Even though wheat is generally grown in water stress-prone parts of the world, soil water potential strongly affects seedling emergence (Hanson *et al.* 1982). In particular, wheat seedlings water-stressed in darkness with 20% PEG 6000, presented lower shoot height, fresh weight and total protein concentration than control ones (Barassi *et al.* 1980). However partially, the negative effects of saline stress (Creus *et al.* 1997) and water stress on shoot growth were attenuated in seedlings previously inoculated with *Azospirillum* (Alvarez *et al.* 1996; Creus *et al.* 1997; Pereyra *et al.* 2006), where a noticeable improvement in the plant water status was also evident (Creus *et al.* 1997). The beneficial *Azospirillum*-wheat association was not hampered by the presence of Tebuconazole, even when exposed to 20% PEG 8000 solutions (Pereyra *et al.* 2009).

In maize, the rapid dehydration of the seedling is associated to the relative small size of their root (Cutforth *et al.* 1986). Roots from 5-days-old seed-inoculated seedlings grown in hydroponics showed a 63% increase in dry weight and a 50% increase in the surface. In addition, the coleoptiles dry weight and water content increased 52 and 22% in inoculated plants (Casanovas *et al.* 2000). *Azospirillum* seed inoculation improved maize seedlings relative water content and water content when grown for 15 days at 75% water supply reduction.

Azospirillum-grasses association in the field

We studied the connections between water relations and the yield in *A. brasilense* Sp245-inoculated wheat suffering drought during anthesis (Creus *et al.* 2004). A lesser loss (16.9%) of grain yield was obtained in *Azospirillum*-inoculated plants suffering drought. The relative increase observed was mainly due to the bacterial effect on the number of grains per square meter (Creus *et al.* 2004). We also studied the mineral content of these grains. They showed higher Mg, Ca and K contents. Neither drought nor inoculation changed grain P, Cu, Fe and Zn contents. In this sense, inoculation would be increasing the cost-benefit ratio, as the *Azospirillum* effect could promote not only a higher grain yield but also a higher seed quality. For example, a higher than normal concentration of Ca in seeds, as that promoted by *Azospirillum* inoculated in water stressed plants, could mean future seedlings with better performance under drought. As it was mentioned above, *A. brasilense* Az 39 INTA initially isolated from Argentine pampas, has been described as the strain with the best performance in *Azospirillum*-based inoculants in Argentina (Díaz-Zorita and Fernández Canigia 2008). It has shown a high survival rate in seeds up to a year, and no side effects when the seeds were previously treated with Carbendazim+Thiram; Tebuconazole or Triticonazole (Díaz-Zorita *et al.* 2004). The inoculums' formulation and application technology are crucial for the development of commercial *Azospirillum* inoculants. Up to now, relatively few studies have addressed the application of *Azospirillum* technology under practical agricultural conditions (Díaz-Zorita and Fernández Canigia 2008). Puente *et al.* (2005) found that *A. brasilense* Az39 INTA inoculated wheat showed an increased grain yield, with an increase in the number of tillers and spikelets and in the roots dry weight at harvest. As it was mentioned Rodríguez Cáceres *et al.* (1996) suggested that *Azospirillum*-inoculated wheat performance in the field depends upon soil fertility and water availability. They also suggested that inoculation might provide superior results when water is a limiting factor. Other numerous field studies on drought-*Azospirillum* inoculated- wheat performance were reported by García de Salamone and Monzón de Asconegui (2008). Hydric stress was not analyzed as an independent factor, so although superior yields were detected in inoculated plants, these authors did not find a self explained unique mechanism.

Maize yield at harvest is highly dependent on the water supply from 15 days before to 20 days after flowering. Water stress at this phenological stage impairs the reproductive yield, a variable closely related to both the number of grains per surface unit and the final grain weight (Grant *et al.* 1989; Fuad Hassan *et al.* 2008). Following a 75% restriction in the water supply during flowering, maize plants grown from *Azospirillum* inoculated seeds had better water status, higher stomatal resistance, chlorophyll content, photosynthetic rate, and free proline content, than the autoclaved bacteria inoculated controls. The physiological improvement of maize plants due to *Azospirillum* inoculation, could account for the amelioration of the harmful effects of drought during the flowering period in both grain yield and harvest index (Casanovas *et al.* 2003).

Mechanisms proposed to cope with water stress

Despite the numerous studies on physiology and molecular biology of *Azospirillum*, there is no definite agreement on exactly how the bacteria affect plant growth, which mechanisms are involved, or if there is one major mechanism responsible for the observed effects on plant growth, particularly, on plant yield (Bashan *et al.* 2004). These questions are thus far the driving force in the *Azospirillum* research field.

The production of phytohormones has been proposed as the key feature of this bacterium and the cause of the altered metabolism and morphology of the plant, yielding better mineral and water absorption (Dobelaere *et al.* 1999). The most apparent outcome after inoculation is the production of morphological changes in the root system. Inoculated plants larger roots would absorb minerals and water better (Okon 1985). The increased root development leads to an increased root surface that could improve plant nutrition and thus would be a key factor for plant growth promotion by PGPR in general. In this sense, developmental changes promoted in roots must be triggered prior to the changes in uptake of nutrients. This widely accepted hypothesis also states that nutrient uptake would be increased over time together with increased root surface. In this view, nutritional improvement by PGPR would be an indirect consequence

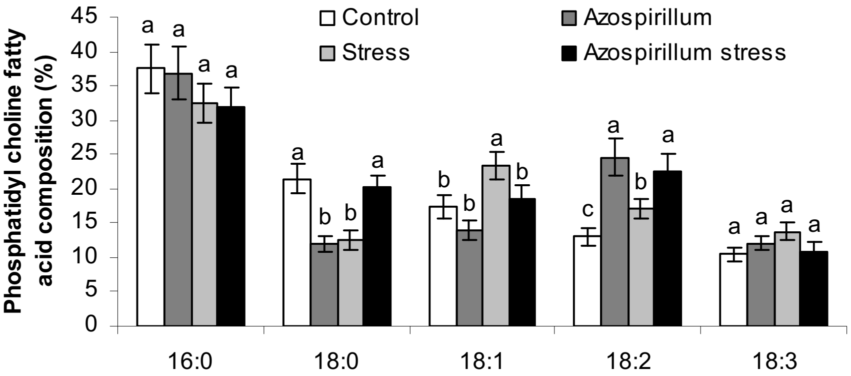

Fig. 2 Fatty acid composition (percentages) in phosphatidylcholine extracted from *Azospirillum*-inoculated wheat seedlings roots grown under normal or water stress conditions. Seedlings were grown in the dark and inoculated either with previously autoclaved *A. brasilense* Sp245 cells (control), or with 10^7 live bacteria per plant. Each of these groups was then grown for 2 days, either in sterile distilled water or in 20% PEG 8000 (stress).

of their effect on root development (Mantelin and Touraine 2004). Nevertheless, direct effects on root transport systems cannot be ruled out. Bertrand *et al.* (2000) showed that an *Achromobacter* sp. enhanced NO_3^- uptake rate per unit of root area in *Brassica napus* roots, and Saubidet *et al.* (2002) reported that the inoculation with *A. brasilense* increased the N content of wheat plants. Our results showed that inoculated wheat seedlings had larger roots than non inoculated controls either in well irrigated conditions or when exposed to 20% PEG 8000 solutions (Pereyra *et al.* 2006, 2009).

It was proposed bacterial direct effects on plant cell membranes as an alternative mechanism. Bashan *et al.* (1992) showed that soybean (*Glycine max*) and cowpea (*Vigna unguiculata*) inoculated with *A. brasilense* enhance H^+ extrusion from their roots compared to the normal extrusion occurred in non-inoculated plants. After nine hours of transferring cowpea plants growing in hydroponic solutions to a new one the pH of the media decreased from 6 units of pH to the range of 4.13 ± 0.19 units, providing more evidence about a direct effect on root cell membranes. The bacteria affect several plant metabolic pathways, including cell membrane activity. To shed light on the possible relationship between the alleviation effects of water stress on wheat, and the lipid composition of roots, we studied *Azospirillum*-inoculated wheat seedlings root phospholipids (PL) composition, fatty acid (FA) distribution profiles, and degree of unsaturation of major PL classes, from seedlings growing in darkness under osmotic stress (Pereyra *et al.* 2006). When both *Azospirillum* inoculation and water stress treatments were combined, the distribution of the major PL classes (phosphatidylcholine (PC) and phosphatidylethanolamine (PE) remained unaltered, while FA distribution profiles changed in both PC and PE. We concluded that *Azospirillum* inoculation could contribute to protect wheat seedlings from water stress through changes in the FA distribution profiles of the major PC and PE root PL (Pereyra *et al.* 2006). Other unpublished experiments performed in our laboratory confirmed that among the FA affected by inoculation followed by stress, the linoleic acid (18:2) content of PC increased the most (**Fig. 2**). As was mentioned, many studies have suggested the involvement of auxins produced by *Azospirillum* on the root morphology (Bashan *et al.* 2004). These hormones are involved in the expression of ER-bound $\Delta 12$ desaturase, a key enzyme in the synthesis of 18:2. As a matter of speculation, this FA could be contributing to regulate the activity of key membrane-associated enzymes such as ATPases via modification of their lipid microenvironment (Palta 1990). Plasma membrane H^+-ATPases pump protons from the cytoplasm to the apoplastic space, where extensive acidification is believed to contribute to cell wall loosening, a prerequisite for cell growth (Rober-Kleber *et al.* 2003).

Plants have developed mechanisms to response to adverse environmental conditions that in some instances permit to repair the damage caused by stress. The maintenance of tissue turgor pressure is essential to several crucial processes upon which plant life depends. Higher plants have developed adaptive mechanisms to cope with water deficit. One of the most recognized mechanisms is osmotic adjustment, which involves active solute accumulation in plant tissues thus enabling plants to extract water at low soil water potential and maintaining cell turgor (Morgan 1984). Could it be possible that *Azospirillum* inoculation affect the stress tolerance mechanisms that trigger plants under stress? In the search for the answer to this question, we developed assays to measure the production of osmolites in root and leaf of inoculated maize. We demonstrated an improved water status and both foliar and radical high free proline concentrations in inoculated maize seedling with *A. brasilense* Sp245 (Casanovas *et al.* 2002). Seedlings also showed increased root growth, total aerial biomass and foliar area. Proline is usually considered as an osmoprotectan agent and their accumulation appears to render membranes more enduring (Sairam and Saxena 2000). However, some authors suggested that proline accumulation in vegetable tissues could be only useful as possible drought injury sensor instead of its role in stress tolerance mechanism (Vartaniam *et al.* 1992; Zlatev and Stoyanov 2005). In addition, proline is also known to be involved in reducing the oxidative damage by scavenging and/or reducing the free radicals, involved in tolerance mechanism against oxidative stress, which is in addition, the main strategy of plant to avoid detrimental effects of water stress (Vendruscolo *et al.* 2007).

On the other hand, in a hydroponic system, were no nutrients were present, *A. brasilense* Sp245 inoculation could also partially reverse the negative effects that water stress had on wheat seedlings, as it was observed in the growth rate of coleoptiles (Alvarez *et al.* 1996). Furthermore, as a correlation between coleoptile length and osmoregulation among wheat genotypes has been reported (Morgan 1988), we studied the impact of *Azospirillum*-inoculation on plant water relations (Creus *et al.* 1998). An overall view of these properties can be obtained in the same tissue by pressure-volume curves (Hellkvist *et al.* 1974). In this sense, the ability of a given plant cell to tolerate a restricted water supply depends on three known physiological mechanisms of adaptation: i) active or passive solute accumulation in vacuoles, ii) changes in cell wall elasticity, and iii) changes in the relative partitioning of water into apoplastic and symplastic fractions (Girma and Krieg 1992). We found differential effects of *Azospirillum* on the wheat seedlings' capability to withstand water stress between different genotypes (Creus *et al.* 1998). Nevertheless, results were consistent with a better water status in *Azospirillum* inoculated wheat seedlings under water stress were both effects on cell wall elasticity and/or apoplastic water were evident (Creus *et al.* 1998). Additionally, the better water status (significantly higher water content, relative water content, water potential, apoplastic water fraction, and lower cell wall modulus of elasticity values) and the additional elastic adjustment found in *Azospirillum* inoculated wheat plants in the field (Creus *et al*. 2004) could be crucial in promoting higher grain yield and mineral quality at harvest, particularly when drought strikes during anthesis.

Biotic and abiotic factors that affect cell wall metabolism will influence its dynamic (i.e. expansion rate and cell growth). We reported that *A. brasilense* induced a more elastic cell wall and a higher apoplastic water fraction in wheat coleoptile and flag leaf (Creus *et al*. 2004). These biophysical characteristics account for an improved response to stress and could permit increased growth even in water stressed plants. Knowledge on the biochemical effects the bacteria could elicit into plant cell wall and how these responses could change the hypocotyl physiology is still scarce. Our first results showed that the cell wall is a target for *Azospirillum* growth promotion (Creus *et al*. 2008; Pereyra *et al*. 2010).

Yet, Cassán *et al*. (2009) proposed another possible mechanism involved in coping water stress. They showed that *A. brasilense* Az39 was capable of producing cadaverine in chemically defined medium and that when present in rice seedling roots it promoted root growth and helped mitigate osmotic stress. They speculated that these effects were due in part to cadaverine production. Polyamines are considered as plant growth regulating compounds; and among them, cadaverine has been correlated with root growth promotion or osmotic stress mitigation in some plant species.

It was mentioned above that no unique and definitive mechanisms could be ascribed to the improved performance of grasses crops under stress. The best explain that was hypothesized since almost twenty years ago and is still alive is the Additive Hypothesis that considers multiple mechanisms in the intimate association between roots and bacteria which operate simultaneously or in succession (Bashan and Levanony 1990). The sum of their activities, when induced under appropriate environmental conditions, results in the observed changes in plant growth and crop yield.

CONCLUSION AND PERSPECTIVES

Great efforts all around the world are being made in many scientific programs in order to develop new crop varieties with enhanced drought and salt tolerance and better nutritional value through the introduction of desirable traits either by conventional breeding or genetic modification. However, the focus generally lay on the plant moiety, ignoring what is occurring with the other moiety of the interaction: the microbial communities of the soil. The important role of rhizobacteria that interact with plant roots and influence plant health, productivity and biodiversity must be included.

Traditional microbial population studies, based on identification, quantification, and the measurement of processes that occur in the rhizosphere, are often difficult or tedious. Similarly, the collection of relevant samples or the simulation of natural conditions in the laboratory can be problematical. However, with the array of molecular techniques that are becoming available, significant improvements in our understanding of rhizosphere microbial communities and processes are anticipated (Barea *et al*. 2005; Johnson *et al*. 2005). Currently, much expectation is on the almost completed genomic sequencing projects of *A. brasilense* Sp245 in U.S.A. and *A. lipoferum* 4B in France. The genomes of other diazotrophic PGPR, like *Azoarcus* sp. BH52, *Herbaspirillum seropedicae* and *Gluconacetobacter diazotrophicus* were recently completed enabling new insights and starting points of even more advanced studies.

Semi-arid conditions make survival difficult for the introduced bacteria. Harsh conditions, including frequent droughts, lack of sufficient irrigation, high salinity and soil erosion, may quickly diminish the population of any bacteria introduced into the soil unless precautions are taken to select the proper inoculant and provide irrigation concomitant with inoculation. However, in agricultural systems in developing countries, beneficial microorganisms may make the greatest contribution, if inexpensive and easy-to-use formulations can be developed (Bashan 1998). *Azospirillum*, as the most studied PGPR (Bashan *et al*. 2004), could provide valuable information that could help to expand its agrobiotechnological applications, as well as to a better understanding of other potentially useful microbial-plant associations.

The *Azospirillum*-based product application in agriculture is supported mainly by their effect as plant growth promoter and their improvement of nutrient assimilation. The adoption of this inoculation practice in association with recommended doses of fertilizers improve the crop tolerance to abiotic stresses without loss of productivity (Divan Baldani *et al*. 2008).

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REFERENCES

Abeles FB, Morgan PW, Salveit ME Jr. (1992) *Ethylene in Plant Biology* (2nd Edn), Academic Press, New York, 414 pp

Adesemoye AO, Torbert III HA, Kloepper JW (2008) Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. *Canadian Journal of Microbiology* **54**, 876-886

Alvarez MI, Sueldo RJ, Barassi CA (1996) Effect of *Azospirillum* on coleoptile growth in wheat seedlings under water stress. *Cereal Research Communications* **24**, 101-107

Arsac JF, Lamothe C, Mulard D, Fages H (1990) Growth enhancement of maize (*Zea mays* L.) through *Azospirillum lipoferum* inoculation: effect of plant genotype and bacteria concentration. *Agronomie* **10**, 649-654

Bacilio M, Rodríguez H, Moreno M, Hernández JP, Bashan Y (2004) Mitigation of salt stress in wheat seedlings by a gfp-tagged *Azospirillum lipoferum*. *Biology and Fertility of Soils* **40**, 188-193

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology* **57**, 233-266

Bakanchikova TI, Lobanok EV, Pavlova-Ivanova LK, Redkina TV, Nagapetyan ZA, Majsuryan AN (1993) Inhibition of tumor formation process in dicotyledonous plants by *Azospirillum brasilense* strains. *Mikrobiologiya* **62**, 515-523

Baldani VLD, Baldani JI, Döbereiner J (1983) Effects of *Azospirillum* inoculation on root infection and nitrogen incorporation in wheat. *Canadian Journal of Microbiology* **29**, 924-929

Baldani VLD, Baldani JI, Döbereiner J (1987) Inoculation of field-grown wheat (*Triticum aestivum*) with *Azospirillum* spp. in Brazil. *Biology and Fertility of Soils* **4**, 37-40

Barassi CA, Crupkin M, Sueldo RJ, Invernati A (1980) Osmotic stress in coleoptiles and primary leaves of wheat. *Journal of Experimental Botany* **125**, 1565-1572

Barassi CA, Sueldo RJ, Creus CM, Carrozzi L, Casanovas EM, Pereyra MA (2007) *Azospirillum* spp., a dynamic soil bacterium favouring vegetable crop production. *Dynamic Soil, Dynamic Plant* **1 (2)**, 68-82

Barbieri P, Galli E (1993) Effect on wheat root development of inoculation with an *Azospirillum brasilense* mutant with altered indole-3-acetic acid production. *Research in Microbiology* **144**, 69-75

Barea JM, Brown ME (1974) Effects on plant growth produced by *Azotobacter paspali* related to synthesis of plant growth regulating substances. *Journal of Applied Bacteriology* **40**, 583-593

Barea JM, Pozo MJ, Azcón R, Azcón-Aguilar C (2005) Microbial co-operation in the rhizosphere. *Journal of Experimental Botany* **56**, 1761-1778

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. *Biotechnology Advances* **16**, 729-770

Bashan Y, Alcaraz-Melendez L, Toledo G (1992) Responses of soybean and cowpea root membranes to inoculation with *Azospirillum brasilense*. *Symbiosis* **13**, 217-228

Bashan Y, de-Bashan LE (2002) Protection of tomato seedlings against infection by *Pseudomonas syringae* pv. tomato by using the plant growth-promoting bacterium *Azospirillum brasilense*. *Applied and Environmental Microbiology* **68**, 2637-2643

Bashan Y, Hartmann A (2009) Ecology and application of *Azospirillum* and other plant growth-promoting bacteria (PGPB). *European Journal of Soil Biology* **45 (Special Issue)**, 1-2

Bashan Y, Holguin G (1997) *Azospirillum*-plant relationships: environmental and physiological advances (1990–1996). *Canadian Journal of Microbiology* **43**, 103-121

Bashan Y, Holguin G, de-Bashan LE (2004) *Azospirillum*–plant relationships: Physiological, molecular, agricultural, and environmental advances (1997–2003). *Canadian Journal of Microbiology* **50**, 521-577

Bashan Y, Levanony H (1990) Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture. *Canadian Journal of Microbiology* **36**, 591-608

Bashan Y, Singh CS, Levanony H (1989) Contribution of *Azospirillum brasilense* Cd to growth of tomato seedlings is not through nitrogen fixation. *Canadian Journal of Botany* **67**, 2429-2434

Baudoin E, Lerner A, Mirza MS, El Zemrany H, Prigent-Combaret C, Jurkevich E, Spaepen S, Vanderleyden J, Nazaret S, Okon Y, Moënne-Loccoz Y (2010) Effects of *Azospirillum brasilense* with genetically modified auxin biosynthesis gene *ipdC* upon the diversity of the indigenous microbiota of the wheat rhizosphere. *Research in Microbiology* **161**, 219-226

Beauchamp CJ (1993) Mode of action of plant growth-promoting rhizobacteria and their potential use as biological control agents. *Phytoprotection* **71**, 19-27

Beligni MV, Lamattina L (2001) Nitric oxide: A non-traditional regulator of plant growth. *Trends in Plant Science* **6**, 508-509

Bertrand H, Plassard C, Pinochet X, Touraine B, Normand P, Cleyet-Marel JC (2000) Stimulation of the ionic transport system in *Brassica napus* by a plant growth-promoting rhizobacterium (*Achromobacter* sp.). *Canadian Journal of Microbiology* **46**, 229-236

Boddey RM, Jantalia CP, Zotarelli L, Okito A, Alves BJR, Urquiaga S, Amado TJC (2008) Techniques for the quantification of plant-associated biological nitrogen fixation. In: DD Samson, BM Chimphango, AJ Valentine, C Elmerich, WE Newton (Eds) *Biological Nitrogen Fixation: Towards Poverty Alleviation through Sustainable Agriculture*, Springer, Berkeley, pp 37-41

Boyer JS (1982) Plant productivity and environment. *Science* **218**, 443-448

Brown ME (1972) Plant growth substances produced by microorganisms of soil and rhizosphere. *Journal of Applied Bacteriology* **43**, 443-451

Caballero-Mellado J, Carcano-Montiel MG, Mascarua-Esparza MA (1992) Field inoculation of wheat (*Triticum aestivum*) with *Azospirillum brasilense* under temperate climate. *Symbiosis* **13**, 243-253

Capelletti JP, García de Salamone IE, Ventimiglia L (2004) Colonización rizosférica con bacterias microaerofílicas, longitud de raíces y rendimiento en grano de plantas de *Zea mays* inoculadas con dos inoculantes comerciales de *Azospirillum* spp. In: Monzón de Asconegui M, García de Salamone IE, Miyazaki S (Eds) *Biología del Suelo. Transformación de la Materia Orgánica. Usos y Biodiversidad de los organismos edáficos*, FAUBA, Buenos Aires, pp 201-205

Casanovas EM, Barassi CA, Sueldo RJ (2000) *Azospirillum* inoculation of maize seed during imbibition. *Cereal Research Communications* **28**, 25-32

Casanovas EM, Barassi CA, Sueldo RJ (2002) *Azospirillum* inoculation mitigates water stress effects in maize seedlings. *Cereal Research Communications* **30**, 343- 350

Casanovas EM, Barassi CA, Andrade FH, Sueldo RJ (2003) *Azospirillum*-inoculated maize plant responses to irrigation restraints imposed during flowering. *Cereal Research Communications* **31**, 395-402

Cassán F, Maiale S, Masciarelli O, Vidal A, Luna V, Ruiz O (2009) Cadaverine production by *Azospirillum brasilense* and its possible role in plant growth promotion and osmotic stress mitigation. *European Journal of Soil Biology* **45 (Special Issue)**, 3-8

Compant S, Duffy B, Nowak J, Clement C, Ait Barka E (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology* **71**, 4951-4959

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

Creus CM (1997) Growth and water relations under abiotic stress of *Azospirillum brasilense*-inoculated *Triticum sp.* [Spanish]. PhD thesis, Faculty of Agricultural Sciences, University of Mar del Plata, Argentina, 162 pp

Creus CM, Sueldo RJ, Barassi CA (1996) *Azospirillum* inoculation in pre-germinating wheat seeds. *Canadian Journal of Microbiology* **42**, 83-86

Creus CM, Sueldo RJ, Barassi CA (1997) Shoot growth and water status in *Azospirillum*-inoculated wheat seedlings grown under osmotic and salt stresses. *Plant Physiology and Biochemistry* **35**, 939-944

Creus CM, Sueldo RJ, Barassi CA (1998) Water relations in *Azospirillum*-inoculated wheat seedlings under osmotic stress. *Canadian Journal of Botany* **76**, 238-244

Creus CM, Sueldo RJ, Barassi CA (2004) Water relations and yield in *Azospirillum*-inoculated wheat exposed to drought in the field. *Canadian Journal of Botany* **8**, 273-281

Creus CM, Pereyra MA, Molina-Favero C, Ramella NA, Casanovas EM, Pereyra CM, Arruebarrena-Di Palma A, Lamattina L, Sueldo RJ, Barassi CA (2008) La pared celular como target en la promoción del crecimiento de las plantas por *Azospirillum.* In: Cassán FD, García de Salamone I (Eds) *Azospirillum sp.: Cell Physiology, Plant Interactions and Agronomic Research in Argentina* (1st Edn), Asociación Argentina de Microbiología, Buenos Aires, pp 95-110

Cutforth HM, Shaykewich CF, Cho CM (1986) Effect of soil water and temperature on corn (*Zea mays* L.) root growth during emergence. *Canadian Journal of Soil Science* **66**, 51-58

Dekhil SB, Cahill M, Stackebrandt E, Sly Li (1997) Transfer of *Conglomeromonas largomobilis* subsp. *largomobilis* to the genus *Azospirillum* as *Azospirillum largomobile* comb. nov., and elevation of *Conglomeromonas largomobilis* subsp. *parooensis* to the new type species of *Conglomeromonas, Conglomeromonas parooensis* sp. nov. *Systematics and Applied Microbiology* **20**, 72-77

Delvasto P, Valverde A, Ballestera A, Igual JM, Muñoz JA, González F, Blázquez ML, García C (2006) Characterization of brushite as a re-crystallization product formed during bacterial solubilization of hydroxyapatite in batch cultures. *Soil Biology and Biochemistry* **3**, 2645-2654

Díaz-Zorita M, Puente M, Perticari A (2004) Inoculación de trigo con *Azospirillum brasilense*: II. Aplicación combinada con fungicidas. In: Monzón de Asconegui M, García de Salamone IE, Miyazaki S (Eds) *Biología del Suelo. Transformación de la Materia Orgánica. Usos y Biodiversidad de los Organismos edáficos*, FAUBA, Buenos Aires, pp 207-212

Díaz-Zorita M, Fernandez Canigia MV (2008) Análisis de la producción de cereales inoculados con *Azospirillum brasilense* en la República Argentina. In: Cassán FD, García de Salamone I (Eds) *Azospirillum sp.: Cell Physiology, Plant Interactions and Agronomic Research in Argentina* (1st Edn), Asociación Argentina de Microbiología, Buenos Aires, pp 153-164

Divan Baldani VL, Silva Ferreira J, dos Santos Teixeira KR, Baldani JI, Massena Reis V (2008) Inoculants based on nitrogen-fixing bacteria *Azospirillum* spp and their application in tropical agriculture. In: Cassán FD, García de Salamone I (Eds) *Azospirillum sp.: Cell Physiology, Plant Interactions and Agronomic Research in Argentina* (1st Edn), Asociación Argentina de Microbiología, Buenos Aires, pp 225-236

Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Vanderleyden J, Dutto P, Labandera-Gonzalez C, Caballero-Mellado J, Aguirre JF, Kapulnik Y, Brener S, Burdman S, Kadouri D, Sarig S, Okon Y (2001) Responses of agronomically important crops to inoculation with *Azospirillum. Australian Journal of Plant Physiology* **28**, 871-879

Dobbelaere S, Croonenborghs A, Thys A, Vande Broek A, Vanderleyden J (1999) Phytostimulatory effect of *Azospirillum brasilense* wild type and mutant strains altered in IAA production in wheat. *Plant and Soil* **212**, 155-164

Döbereiner J, Day JM (1976) Associative symbioses in tropical grasses: characterization of microorganisms and dinitrogen-fixing sites. In: Newton WE, Nyman CJ (Eds) *Proceedings of the 1st International Symposium on Nitrogen Fixation*, Washington State University Press, Pullman, USA, pp 518-538

Doran JW (2002) Soil health and global sustainability: Translating science into practice. *Agriculture, Ecosystems and Environment* **88**, 119-127

Eckert B, Weber OB, Kirchof G, Halbritter A, Stoffels M, Hartmann A (2001) *Azospirillum doebereinerae* sp. nov., a nitrogen-fixing bacterium associated with the C4-grass *Miscanthus*. *International Journal of Systematic Evolution and Microbiology* **51**, 17-26

Fages J (1994) *Azospirillum* inoculants and field experiments. In: Okon Y (Ed) *Azospirillum/Plant Associations*, CRC Press, Boca Raton, Florida, pp 87-110

Fallik E, Sarig S, Okon Y (1994) Morphology and physiology of plant roots associated with *Azospirillum*. In: Okon Y (Ed) *Azospirillum/Plant Associations*. CRC Press, Boca Raton, Florida, pp 77-85

Fuad-Hassan A, Tardieu F, Turc O (2008) Drought-induced changes in anthesis-silking interval are related to silk expansion: A spatio-temporal growth analysis in maize plants subjected to soil water deficit. *Plant Cell and Environment* **31**, 1341-1360

Gan Y, Stobbe EH, Moes J (1992) Relative date of wheat seedling emergence and its impact on grain yield. *Crop Science* **32**, 1275-1281

García de Salamone IE, Döbereiner J (1996) Maize genotype effects on the response to *Azospirillum* inoculation. *Biology and Fertility of Soils* **21**, 193-196

García de Salamone IE, Monzón de Asconegui MA, Zawosnik M (1992) Respuesta de tres híbridos comerciales de maíz a la inoculación con *Azospirillum. Turrialba* **42**, 359-364

García de Salamone IE, Monzón de Asconegui MA (2008) Ecofisiología de la respuesta a la inoculación con *Azospirillum* en cultivos de cereales. In: Cassán FD, García de Salamone I (Eds) *Azospirillum sp.: Cell Physiology, Plant Interactions and Agronomic Research in Argentina* (1st Edn), Asociación Argentina de Microbiología, Buenos Aires, pp 207-224

Genoud T, Métraux JP (1999) Crosstalk in plant cell signaling: Structure and function of the genetic network. *Trends in Plant Science* **4 (12)**, 503-507

Girma FS, Krieg DR (1992) Osmotic adjustment in sorghum I. Mechanisms of diurnal osmotic potential changes. *Plant Physiology* **99**, 577-582

Glick BR (1995) The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* **41**, 109-117

Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *Journal of Theoretical Biology* **190**, 63-68

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by bacterial ACC deaminase. *Critical Review of Plant Science* **26**, 227-242

Grant RF, Jackson BS, Kiniry JR, Arkin GF (1989) Water deficit timing effects on yield components in maize. *Agronomy Journal* **81**, 61-65

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: Commonalities and distinctions in the plant bacterium signaling process. *Soil Biology and Biochemistry* **37**, 395-412

Hanson H, Borlaug NE, Anderson RG (1982) La importancia del trigo y sus principales características. In: Centro Internacional de Mejoramiento de Maíz y Trigo (Eds) *Trigo en el Tercer Mundo*, México, pp 1-12

Hellkvist J, Richards GP, Jarvis PG (1974) Vertical gradients of water potential and tissue water relations in Sitka spruce trees measured with the pressure chamber. *Journal of Applied Ecology* **11**, 637-668

Hungría M, Campo RJ, Souza EM, Pedrosa FO (2010) Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant and Soil* **331**, 413-425

Jacobsen CS (1997) Plant protection and rhizosphere colonization of barley by seed inoculated herbicide degrading *Burkholderia* (*Pseudomonas*) *cepacia* DBO1(pRO101) in 2,4-D contaminated soil. *Plant and Soil* **189**, 139-144

Jain DK, Patriquin DG (1984) Root hair deformation, bacterial attachment, and plant growth in wheat-*Azospirillum* associations. *Applied and Environmental Microbiology* **48**, 1208-1213

Johnson DR, Lee PKH, Holmes VF, Fortin AC, Alvarez-Cohen L (2005) Transcriptional expression of the *tceA* gene in a *Dehalococcoides*-containing microbial enrichment. *Applied and Environmental Microbiology* **71**, 7145-7151

Kapulnik Y, Gafny R, Okon Y (1985) Effect of *Azospirillum* spp. inoculation on root development and NO_3^- uptake in wheat (*Triticum aestivum* cv. Miriam) in hydroponic systems. *Canadian Journal of Botany* **63**, 627-631

Kennedy IR, Choudhury ATMA, Kecskés ML (2004) Non-symbiotic bacterial diazotrophs in crop-farming systems: Can their potential for plant growth promotion be better exploited? *Soil Biology and Biochemistry* **36**, 1229-1244

Khammas KM, Ageron E, Grimont PA, Kaiser P (1989) *Azospirillum irakense* sp. nov., a nitrogen-fixing bacterium associated with rice roots and rhizosphere soil. *Research in Microbiology* **140**, 679-693

Kloepper JW, Schroth MN (1978) Plant growth-promoting rhizobacteria on radishes. In: Station de Pathologie Végétale et de Phytobactériologie, INRA, Angers, France. *Proceedings of the 4th International Conference on Plant Pathogenic Bacteria* 2, 879-882

Kloepper JW, Lifshitz R, Zablotowicz RM (1989) Free-living bacterial inocula for enhancing crop productivity. *Trends in Biotechnology* **7**, 39-44

Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* species. *Phytopathology* **94**, 1259-1266

Kokalis-Burelle N, Kloepper JW, Reddy MS (2006) Plant growth-promoting rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms. *Applied Soil Ecology* **31**, 91-100

Kotb THS,Watanabe T, Ogino Y, Tanji KK (2000) Soil salinization in the Nile Delta and related policy issues in Egypt. *Agricultural Water Manage* **43**, 239-261

Kundu BS, Sangwan P, Sharma PK, Nandwal AS (1997) Response of pearl millet to phytohormones produced by *Azospirillum brasilense*. *Indian Journal of Plant Physiology* **2**, 101-104

Lafond GP, Fowler BD (1989) Soil temperature and water content, seedling depth, and simulated rainfall effects on winter wheat emergence. *Agronomy Journal* **81**, 609-614

Lamattina L, Garcia-Mata C, Graziano M, Pagnussat G (2003) Nitric oxide: The versatility of an extensive signal molecule. *Annual Review of Plant Biology* **54**, 109-136

Lambrecht M, Okon Y, Vande Broek A, Vanderleyden J (2000) Indole-3-acetic acid: A reciprocal signalling molecule in bacteria-plant interactions. *Trends in Microbiology* **8**, 298-300

Levanoni H, Bashan Y, Romano B, Klein E (1989) Ultrastructural localization and identification of *Azospirillum brasilense* Cd on and within root by inmuno-gold labeling. *Plant and Soil* **117**, 207-218

Lin S-Y, Young CC, Hupfer H, Siering C, Arun AB, Chen W-M, Lai W-A, Shen F-T, Rekha PD, Yassin AF (2009) *Azospirillum picis* sp. nov., isolated from discarded tar. *International Journal of Systematic and Evolutionary Microbiology* **59**, 761-765

Maddoni GA, Vilariño P, García de Salamone IE (2004) Dinámica de los nutrintes en el sistema suelo – planta. In: Satorre E, Benech Arnold R, Slafer GA, de la Fuente EB, Miralles DJ, Otegui ME, Savin R (Eds) *Producción de Granos, Bases Funcionales Para su Manejo*, FAUBA, Buenos Aires, pp 441-477

Madkour MA, Smith LT, Smith GM (1990) Preferential osmolyte accumulation: A mechanism of osmotic stress adaptation in diazotrophic bacteria *Applied and Environmental Microbiology* **56 (9)**, 2876-2881

Magalhães FM, Baldani JI, Souto SM, Kuykendall JR Jr., Döbereiner JA (1983) New acid-tolerant *Azospirillum* species. *Anais da Academia Brasileira de Ciências* **55**, 417-430

Mantelin S, Touraine B (2004) Plant growth-promoting bacteria and nitrate availability: Impacts on root development and nitrate uptake. *Journal of Experimental Botany* **55**, 27-34

Mehnaz S, Weselowski B, Lazarovits G (2007a) *Azospirillum canadense* sp. nov., a nitrogen-fixing bacterium isolated from corn rhizosphere. *International Journal of Systematic and Evolutionary Microbiology* **57**, 620-624

Mehnaz S, Weselowski B, Lazarovits G (2007b) *Azospirillum zeae* sp. nov., a diazotrophic bacterium isolated from rhizosphere soil of *Zea mays*. *International Journal of Systematic and Evolutionary Microbiology* **57**, 2805-2809

Molina-Favero C, Creus CM, Lanteri ML, Correa Aragunde N, Lombardo MC, Barassi CA, Lamattina L (2007) Nitric oxide and plant growth promoting rhizobacteria: Common features influencing root growth and development. *Advances in Botanical Research* **46**, 1-33

Molina-Favero C, Creus CM, Simontacchi M, Puntarulo S, Lamattina L. (2008) Aerobic nitric oxide production by *Azospirillum* brasilense Sp245 and its influence on root architecture in tomato *Molecular Plant-Microbe Interactions* **21 (7)**, 1001-1009

Morgan JM (1988) The use of coleoptile responses to water stress to differenttiate wheat genotypes for osmoregulation, growth and yield. *Annals of Botany (London)* **62**, 193-198

Morgan NM (1884) Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiology* **35**, 200-319

Morgestern E, Okon Y (1987) The effect of *Azospirillum brasilense* and auxin on root morphology in seedlings of *Sorghum bicolor* x *Sorghum sudanense*. *Arid Soil Research and Rehabilitation* **1**, 115-127

Murphy DJ, Piffanelli P (1998) Fatty acid desaturases: structure, mechanism and regulation. In: Harwood J (Ed) *Plant Lipid Biosynthesis. Fundamentals and Agricultural Applications*, Society for Experimental Biology Seminar Series 67, Cambridge University Press, England, pp 95-130

Nabti E, Sahnoune M, Ghoul M, Fischer D, Hofmann A, Rothballer M, Schmid M, Hartmann A (2010) 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*. *The Journal of Plant Growth Regulation* **29**, 6-22

Okon Y (1985) *Azospirillum* as a potential inoculant for agriculture. *Trends in Biotechnology* **3**, 223-228

Okon Y, Kapulnik Y (1986) Development and function of *Azospirillum*-inoculated roots. *Plant and Soil* **90**, 3-16

Okon Y, Labandera-González CA (1994) Agronomic applications of *Azospirillum*: An evaluation of 20 years worldwide field inoculation. *Soil Biology and Biochemistry* **26**, 1591-1601

Okon Y, Vanderleyden J (1997) Root-associated *Azospirillum* species can stimulate plants. *ASM News* **63**, 364-370

Oliveira RGB, Drozdowicz A (1987) Inhibition of bacteriocin producing strains of *Azospirillum lipoferum* by their own bacteriocin. *Zentralblatt für Mikrobiologie* **142**, 387-391

Palta JP (1990) Stress interactions at the cellular and membranes levels. *HortScience* **25**, 1377-1381

Patrikin DG, Döbereiner J, Jain DK (1983) Sites and processes of association between diazotrophs and grasses. *Canadian Journal of Microbiology* **29**, 900-915

Pedraza RO, Bellone CH, Carrizo de Bellone S, Fernandes Boa Sorte PM, dos Santos Teixeira KR (2008) *Azospirillum* inoculation and nitrogen fertilization effect on grain yield and on the diversity of endophytic bacteria in the phyllosphere of rice rainfed crop. *European Journal of Soil Biology* **45 (Special Issue)**, 36-43

Peng G, Wang H, Zhang G, Hou W, Liu Y, Wang ET, Tan Z (2006) *Azospirillum melinis* sp. nov., a group of diazotrophs isolated from tropical molasses grass. *International Journal of Systematic and Evolutionary Microbiology* **56**, 1263-1271

Pereyra CM, Ramella NA, Pereyra MA, Barassi CA, Creus CM (2010) Changes in cucumber hypocotyl cell wall dynamics caused by *Azospirillum brasilense* inoculation. *Plant Physiology and Biochemistry* **48**, pp 62-69

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. *European Journal of Soil Biology* **45 (Special Issue)**, 20-27

Pereyra MA, González RL, Creus CM, Barassi CA (2007) Root colonization vs. seedling growth, in two *Azospirillum*-inoculated wheat species. *Cereal Research Communications* **35**, 1621-1629

Pereyra MA, Zalazar CA, Barassi CA (2006) Root phospholipids in *Azospirillum*-inoculated wheat seedlings exposed to water stress. *Plant Physiology and Biochemistry* **44**, 873-879

Puente ME, Holguin G, Glick BR, Bashan Y (1999) Root-surface colonization of black mangrove seedlings by *Azospirillum halopraeferens* and *Azospirillum brasilense* in seawater. *FEMS Microbiology Ecology* **29**, 283-292

Puente M, Montecchia M, Perticari A (2005) Evaluation of *Azospirillum* inoculant strains in wheat. In: *Abstracts 7th International Wheat Conference*, p 211

Puente ML, García JE, Perticari A (2008) Investigación aplicada de *Azospirillum* para su uso como promotor del crecimiento en cultivos de interés agronómico. In: Cassán FD, García de Salamone IE (Eds) *Azospirillum sp.: Cell Physiology, Plant Interactions and Agronomic Research in Argentina* (1st Edn), Asociación Argentina de Microbiología, Buenos Aires, pp 165-177

Ramamoorthy V, Viswanathan R, Raguchander T, Prakasam V, Samiyappan R (2001) Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Protection* **20**, 1-11

Reed MLE, Warner B, Glick BR (2005) Plant growth-promoting bacteria facilitate the growth of the common reed *Phragmites australis* in the presence of copper or polycyclic aromatic hydrocarbons. *Current Microbiology* **51**, 425-429

Reinhold B, Hurek T, Fendrik I, Pot B, Gillis M, Kersters K, Thielemans S, de Ley J (1987) *Azospirillum halopraeferens* sp. nov., a nitrogen-fixing organism associated with roots of kallar grass (*Leptochloa fusca* (L.) Kunth).

International Journal of Systematic Bacteriology **37**, 43-51

Ribaudo CM, Krumpholz EM, Cassán FD, Bottini R, Cantore ML, Curá JA (2006) *Azospirillum* sp. promotes root hair development in tomato plants through a mechanism that involves ethylene. *The Journal of Plant Growth Regulation* **24**, 175-185

Rober-Kleber N, Albrechtová JTP, Fleig S, Huck N, Michalke W, Wagner E, Speth V, Neuhaus G, Fischer-Iglesias C (2003) Plasma membrane H+-ATPase is involved in auxin-mediated cell elongation during wheat embryo development. *Plant Physiology* **131**, 1302-1312

Roberts JL, Roberts E (1939) Auxin production by soil microorganism. *Soil Science* **48**, 135-139

Rodríguez Cáceres EA, González Anta G, López JR, Di Ciocco CA, Pacheco Basurco JC, Parada JL (1996) Response of field-grown wheat to inoculation with *Azospirillum brasilense* and *Bacillus polymyxa* in the semi-arid region of Argentina. *Arid Soil Research and Rehabilitation* **10**, 13-20

Rodríguez Cáceres EA, Di Ciocco CA, Carletti SM (2008) 25 años de investigación de *Azospirillum brasilense* Az 39 en Argentina. In: Cassán FD, García de Salamone IE (Eds) *Azospirillum sp.: Cell Physiology, Plant Interactions and Agronomic Research in Argentina* (1st Edn), Asociación Argentina de Microbiología, Buenos Aires, pp 177-186

Rodriguez H, Fraga T, Bashan Y (2006) Genetics of phosphate solubilization and potential applications for improving plant growth-promoting bacteria. *Plant and Soil* **287**, 15-21

Ryu CM, Farag MA, Hu CH, Reddy MS, Wei HX, Pare PW, Kloepper JW (2003) Bacterial volatiles promote growth in *Arabidopsis*. *Proceedings of the National Academy of Sciences USA* **100**, 4927-4932

Sairam RK, Saxena DC (2000) Oxidative stress and antioxidants in wheat genotypes: Possible mechanism of water stress tolerance. *Journal of Agronomy and Crop Science* **184**, 55-61

Sarig S, Blum A, Okon Y (1988) Improvement of the water status and yield of field-grown grain sorghum (*Sorghum bicolor*) by inoculation with *Azospirillum brasilense*. *The Journal of Agricultural Science* **110**, 271-277

Sarig S, Okon Y, Blum A (1990) Promotion of leaf area development and yield in *Sorghum bicolor* inoculated with *Azospirillum brasilense*. *Symbiosis* **9**, 235-245

Saubidet MI, Fatta N, Barneix AJ (2002) The effect of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization by wheat plants. *Plant and Soil* **245**, 215-222

Shah S, Karkhanis V, Desai A (1992) Isolation and characterization of siderophore, with antimicrobial activity, from *Azospirillum lipoferum* M. *Current Microbiology* **25**, 34-35

Shannon MC (1997) Adaptation of plants to salinity. *Advances in Agronomy* **60**, 75-120

Somers E, Ptacek D, Gysegom P, Srinivasan M, Vanderleyden J (2005) *Azospirillum brasilense* produces the auxin-like phenylacetic acid by using the key enzyme for indole-3-acetic acid biosynthesis. *Applied and Environmental Microbiology* **71**, 1803-1810

Steenhoudt O, Vanderleyden J (2000) *Azospirillum* a free-living nitrogen fixing bacterium closely associated with grasses: Genetic, biochemical and ecological aspects. *FEMS Microbiology Reviews* **24**, 487-506

Sturz AV, Nowak J (2000) Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. *Applied Soil Ecology* **15**, 183-190

Sudhakar P, Gangwar SK, Satpathy B, Sahu PK, Ghosh JK, Saratchandra B (2000) Evaluation of some nitrogen fixing bacteria for control of foliar diseases of mulberry (*Morus alba*). *Indian Journal of Agriculture* **39**, 9-11

Sumner ME (1990) Crop responses to *Azospirillum* inoculation. *Advances in Soil Science* **12**, 53-123

Tapia-Hernández A, Mascarúa-Esparzá MA, Caballero-Mellado J (1990) Production of bacteriocins and siderophore-like activity in *Azospirillum brasilense*. *Microbios* **64**, 73-83

Tarrand JJ, Krieg NR, Döbereiner J (1978) A taxonomic study of the *Spirillum lipoferum* group with description of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasilense* nov. *Canadian Journal of Microbiology* **24**, 967-980

Tien TM, Gaskin MH, Hubbell DH (1979) Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Applied and Environmental Microbiology* **37**, 1016-1024

Timmusk ES, Wagner GH (1999) The plant-growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: A possible connection between biotic and abiotic stress responses. *Molecular Plant-Microbe Interactions* **12**, 951-959

Vande Broek A, Vanderleyden J (1995) The role of bacterial motility, chemotaxis, and attachment in bacteria-plant interactions. *Molecular Plant-Microbe Interactions* **8**, 800-810

Vande Broek A, Dobbelaere S, van der Leyden J, van Dommeles A (2000) *Azospirillum*-plant root interactions: signalling and metabolic interactions. In: Triplett EW (Ed) *Prokariotic Nitrogen Fixation: A Model System for the Analysis of a Biological Process*, Horizon Scientific Press, Wymondham, UK, pp 761-777

van Loon LC (2004) Systemic resistance induced by rhizosphere bacteria**.** *Annual Review of Phytopathology* **36**, 453-483

van Loon LC, Geraats BPJ, Linthorst HJM (2006) Ethylene as a modulator of disease resistance in plants. *Trends in Plant Science* **11**, 184-191

van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. *European Journal of Plant Pathology* **119**, 243-244

Vartanian N, Hervochon P, Marcotte L, Larher F (1992) Proline accumulation during drought rhizogenesis in *Brassica napus* var. *oleifera. Journal of Plant Physiology* **140**, 623-628

Vendruscolo ACG, Schuster I, Pileggi M, Scapim CA, Molinari HBC, Muarur CJ, Vieira LGC (2007) Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *Journal of Plant Physiology* **164**, 1367-1376

Xia Y, Embley TM, O'Donnell AG (1994) Phylogenetic analysis of *Azospirillum* by direct sequencing of PCR-amplified 16S rDNA. *Systematic and Applied Microbiology* **17**, 197-201

Xie CH, Yokota A (2005) *Azospirillum oryzae* sp. nov., a nitrogen-fixing bacterium isolated from the roots of the rice plant *Oryza sativa. International Journal of Systematic and Evolutionary Microbiology* **55**, 1435-1438

Yang J, Kloepper JW, Ryu ChM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science* **14**, 1-4

Young CC, Hupfer H, Siering C, Ho M-J, Arun AB, Lai W-A, Rekha PD, Shen F-T, Hung M-H, Chen W-M, Yassin AF (2008) *Azospirillum rugosum* sp. nov., isolated from oil-contaminated soil. *International Journal of Systematic and Evolutionary Microbiology* **58**, 959-963

Zamudio M, Bastarrachea F (1994) Adhesiveness and root hair deformation capacity of *Azospirillum* strains for wheat seedlings. *Soil Biology and Biochemistry* **26**, 791-797

Zlatev Z, Stoyanov Z (2005) Effects of water stress on leaf water relations of young bean plants. *Journal of Central European Agriculture* **6**, 5-13