Soil Biology & Biochemistry 103 (2016) 117–130

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Review paper

Azospirillum sp. in current agriculture: From the laboratory to the field

Fabricio Cassán [a], [*], Martín Diaz-Zorita [b]

[a] *Laboratorio de Fisiología Vegetal y de la Interacción Planta-Microorganismo, Universidad Nacional de Río Cuarto, Ruta 36, Km 601, Campus Universitario, CP 5800, Río Cuarto, Córdoba, Argentina*
[b] *Technical Development, Monsanto BioAg, Pilar, Buenos Aires, Argentina*

ARTICLE INFO

Article history:
Received 1 June 2016
Received in revised form
16 August 2016
Accepted 17 August 2016

Keywords:
Biological-products
PGPR
Phytohormones
Inoculants
Sustainable agriculture

ABSTRACT

Azospirillum sp., one of the best studied genus of plant growth promoting rhizobacteria, are able to colonize hundreds of plant species and improve their growth, development and productivity. Free nitrogen fixation and additive mechanisms related to the ability of *Azospirillum* sp. to produce phytohormones and other related molecules are proposed for explaining the plant growth promotion effects on inoculated plants, mainly under stressing conditions. Under field evaluations, the benefits of *Azospirillum* sp. inoculation, mostly related with plant attributes defined during early growth, can be related with the increase in the root development enlarging the explored soil volume for nutrients and water acquisition. Recently published reports of *Azospirillum* sp. inoculation of dryland crops showed grain yield responses on winter (14.0%) and summer cereals (9.5%) and also on legumes (6.6%). These responses are barely observed under strong stressful growing conditions (i.e. severe droughts, major nutrients limitations, etc.) and are currently obtained 70% of the time because the complex interaction between the modes of action of *Azospirillum* sp. and plants, the methods of inoculation and diverse crop production conditions. The practice of inoculating with selected strains of *Azospirillum* sp. provides a direct contribution increasing crop yields and enhance the efficacy in the use of production resources with extended benefits to the environment. One of the achievements from the research is the commercial use of azospirilla inoculants in approximately 3.5 million ha, mainly cultivated with cereals in South America. However, more coordinated communication programs of its complementary benefits for the development of sustainable crop production practices are still needed.

Contents

---

[*] Corresponding author.
E-mail address: fcassan@exa.unrc.edu.ar (F. Cassán).

http://dx.doi.org/10.1016/j.soilbio.2016.08.020
0038-0717/

1. *Azospirillum* sp. as model of plant growth promoting rhizobacteria

Azospirillum sp. is one of the best studied genus of plant growth promoting rhizobacteria at present. This microorganism is able to colonize more than one hundred plant species and significantly improves their growth, development and productivity under field conditions (Bashan and de-Bashan, 2010). One of the principal mechanisms proposed for *Azospirillum* sp. to explain plant growth promotion of inoculated plants, has been related to its ability to produce and metabolize several phytohormones and other plant growth regulation molecules (Tien et al., 1979). From a historical perspective, many studies detailing the beneficial effects of inoculation with beneficial rhizobacteria, especially *Azospirillum* sp., have been undertaken and they describe morphological and physiological changes that occur in inoculated plants. However, in many cases the processes, or the compounds responsible for inducing such responses, have not been unequivocally identified and therefore responses are usually considered within a “black box” model which goes beyond the resulting growth promotion due to the presence of only these organisms or active metabolites in the culture medium or plant tissues.

2. *Azospirillum* sp. and it mechanisms to promote plant growth

First mechanism proposed for explaining the capacity of *Azospirillum* sp. to promote the plant growth was the biological nitrogen fixation (Okon et al., 1983). The evidence used to purpose that mechanism was: (1) inoculation, significantly increases the total nitrogen content in shoots and grains; (2) inoculation, commonly reduces the required doses of nitrogen fertilization for many plant species; (3) inoculation contributes to improve the N balance of plants (Kapulnik et al., 1981). Oppositely, Bashan and Holguin (1997) and Bashan and Levanony (1990) showed that the contribution of nitrogen fixation by *Azospirillum* reached less than 20% of the total N increase in the plant. These findings indicated agronomic significance than initially were expected for this bacterial genus (Kennedy et al., 1997). A decade later, new studies showed there is not unequivocal evidence that nitrogen fixation by *Azospirillum* or other free-living nitrogen fixers plays a significant role in agriculture (For details see Giller and Merckx, 2003).

Other mechanisms of plant growth promotion have been studied and proposed for this microbial genus, such as phytohormone and/or siderophore production, phosphate solubilization (Puente et al., 2004), biocontrol of phytopathogens (Bashan and de-Bashan, 2010) and protection of plants against stress like soil salinity or toxic compounds (Creus et al., 1997). Tien et al. (1979) were the first to suggest that *Azospirillum* sp. could enhance plant growth by auxins and particularly indole-3-acetic acid (IAA) production, and subsequent studies showed the capacity of this genus to produce several other phytohormones and plant growth regulators. Although many mechanisms have been described to explain the plant growth promotion by *Azospirillum* sp., one single mechanism is mostly not responsible for the full effect. *Azospirillum* sp. modes of action could be better explained by the “additive hypothesis” which allows explaining the plant growth promoting effects due to inoculation. This hypothesis was suggested more than 20 years ago (Bashan and Levanony, 1990) and considers multiple mechanisms rather than one mechanism participating in the successful association of *Azospirillum* with plants.

Today, after more than eight decades of studies, we know that these rhizobacteria have been correlated with the production of auxins (Prinsen et al., 1993), cytokinins (Tien et al., 1979), gibberellins (Bottini et al., 1989), ethylene (Strzelczyk et al., 1994), and other plant growth regulators, such as abscisic acid (Cohen et al., 2008), nitric oxide (Creus et al., 2005) and polyamines like spermidine, spermine and the diamine cadaverine (Cassán et al., 2009). Several of the biologically active plant regulators produced by *Azospirillum* sp. are summarized and ranked according their effects on plants in Table 1 (adapted from Cassán et al., 2014).

In the case of the most important groups of plant hormones produced by *Azospirillum* sp. such auxins (IAA), cytokinins (Z) and gibberellins (GA_3), their concentration is lower at early exponential growth phase, but increases during the exponential and/or stationary growth phase, because these compounds are continuously accumulated in the culture medium according to a batch fermentation model (Ona et al., 2003; Cassán et al., 2009a, 2010). Considering this fact, under industrial production of inoculants, the bacterial growth and their capacity to accumulate these metabolites in culture medium should alter the behaviour of the bioproduct and its capacity to promote, in a short term way, the plant growth according to the concentration and composition of those phytohormones in the formulation. In this sense, Okon (1982) reported that after seed inoculation the number of viable cells of *Azospirillum* declines very rapidly. So, the short term benefits of inoculation are not strictly related to the presence of the bacterial cells in the inoculant, but are at least in part related to the presence and concentration of phytohormones. So, the final model to explain the positive effects of inoculation with *Azospirillum* sp. a phytohormones-producer plant growth promoting rhizobacteria (PGPR) are summarized and illustrated in Fig. 1.

Table 1
Overview of biologically active plant growth regulators produced *in vitro* by *Azospirillum* sp. based on their class (i.e. biologically active molecules identified from *Azospirillum* sp. liquid cultures by unequivocal methodology like HPLC or, GC-MS) and its hierarchy (i.e. related to the importance of the phytohormonal role in its interaction with the plant considered by the authors based on available evidence) (adapted from Cassán et al., 2014). IAA: indole-3-acetic acid; PAA: phenylacetic acid; IBA: indole-3-butyric acid; NO: nitric oxide; iP: isopentenyl adenine; iPr: isopentenyl adenine riboside; Z: zeatin; t-Zr: trans zeatin riboside; GA3,1, gibberellins n; Et: Ethylene; ABA: abscisic acid; Cad: cadaverine; Spm: spermine; Spd: spermidine; Put: putrescine.

Class	Hierarchy	Molecules	References
Auxins	1st	IAA, PAA, IBA	Prinsen et al. (1993) Martínez-Morales et al. (2003) Somers et al. (2005)
Gibberellins	4th	GA_3, GA_1	Bottini et al. (1989) Piccoli and Bottini (1996)
Cytokinins	3rd	iP, iPr, Z, *t*-Zr	Horemans et al. (1986) Esquivel-Cote et al. (2010)
Ethylene	5th	Et	Strzelczyk et al., 1994
ABA	6th	ABA	Kolb and Martin (1985)
Nitric oxide	2nd	NO	Creus et al. (2005)
Polyamines	7th	Cad, Spm, Spd, Put	Cassán et al. (2009a) Thuler et al. (2003)

2.1. *Effects of Azospirillum sp. on rhizobia-legume symbiosis*

Root nodulating bacteria induce nodule formation on legume

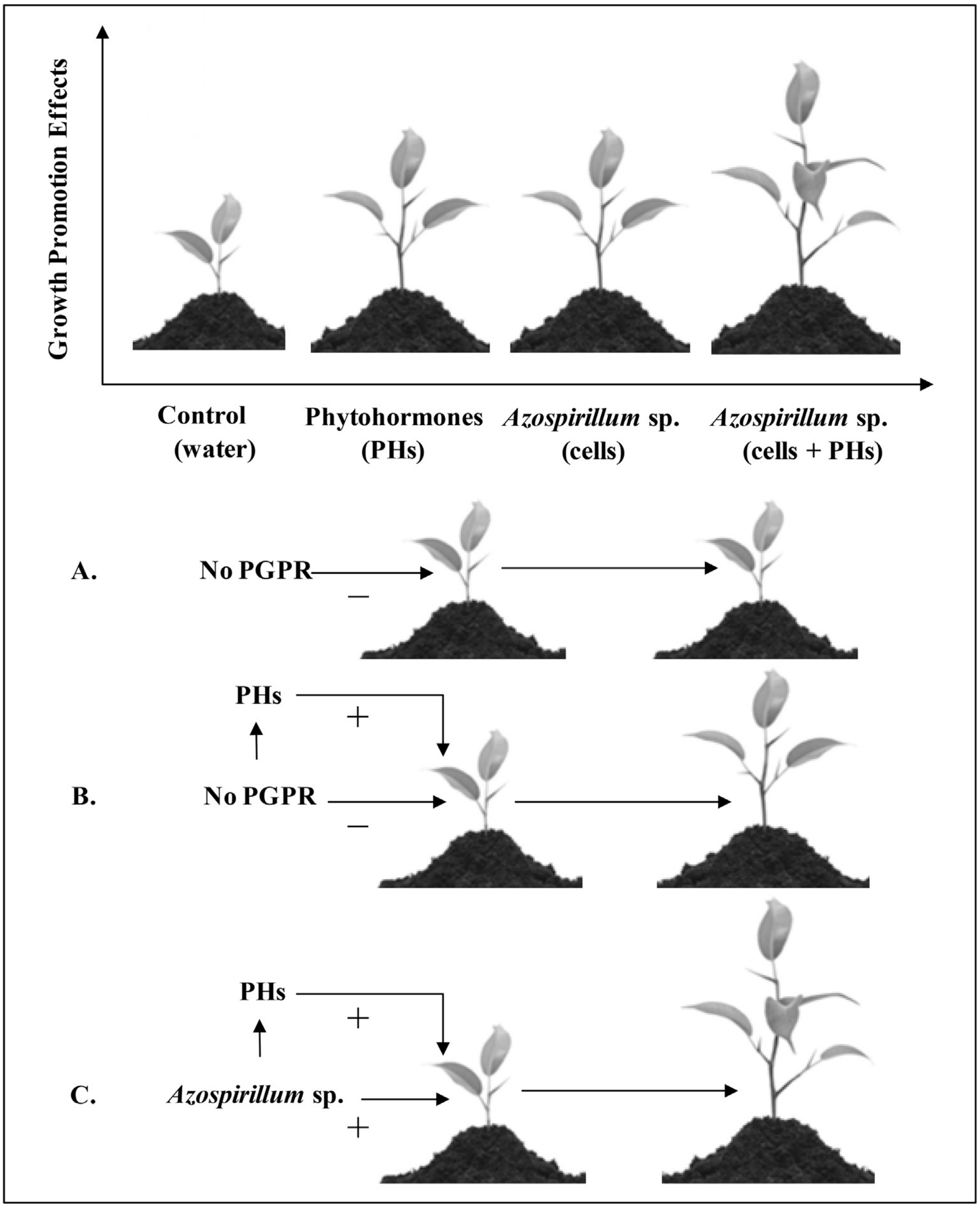

Fig. 1. Illustrative model to explain the positive short-term effects of seeds and seedlings inoculation with *Azospirillum* sp. (a phytohormones-producer PGPR). In the upper side, the model suggest that benefits of inoculation (as plant growth promotion) are not only related to the presence of the bacteria in a product, because the exogenous treatment of seeds or seedlings with similar concentrations of phytohormones, as those produced by *Azospirillum* sp. induces the plant growth promotion effects; however the better response is mostly obtained in presence of both, *Azospirillum* sp. and phytohormones released by the rhizobacteria in culture medium during industrial propagation. A. Inoculation with non benefit rhizobacteria shouldn't induce growth promotion on seeds or seedlings; B. Inoculation with non-benefit, but phytohormones producer rhizobacteria should induces growth promotion (due to the presence of active metabolites); C. Inoculation with *Azospirillum* sp. (phytohormones producer rhizobacteria) induces a higher growth promotion response on seeds or seedlings (Summarized from Tien et al., 1979; Okon, 1982; Cassán et al., 2009a, 2010).

roots and these structures provide the plant with fixed atmospheric nitrogen (Bergersen, 1971). Thiman (1936) proposed that phytohormones such as auxins play an important role in the ontogeny of nodules in rhizobia-legume symbiosis and many studies have indicated that changes in the concentration of IAA or its balance with CK are a prerequisite for nodule organogenesis (Mathesius et al., 1997). Therefore, co-inoculation with phytohormones-producing rhizobacteria can influence the symbiotic outcome by altering the phytohormonal homeostasis. Inoculation of common bean with *A. brasilense* resulted in increased production of plant root flavonoids and enhanced capacity to induce *nod* gene expression in *Rhizobium* (Burdman et al., 1996). The positive effects of *A. brasilense* on legumes growth, nodule organogenesis, flavonoids, and lipochito-oligosaccharide production were assessed in a *Rhizobium*-common bean symbiosis by Dardanelli et al. (2008). Co-inoculation of *Sinorhizobium meliloti* (inefficient IAA producer) with *A. brasilense* (efficient IAA producer) on alfalfa seeds significantly increased the number of root nodules in the primary root and this increase was correlated with the inoculum size. The response could be mimicked by the addition of exogenous IAA (Schmidt et al., 1988). Direct evidence of the role of IAA-promoting effects in co-inoculation studies of *A. brasilense* and *R. etli* on common bean was also provided by Remans et al. (2008a, 2008b) with the use of the *ipdC* knockout mutant of *A. brasilense*. *A. brasilense* Sp7 was recently compared with several mutants affected in production of IAA and nitric oxide for their effects on the symbiosis between *Vicia sativa* spp. nigra (vetch) and *Rhizobium leguminosarum* bv. viciae (Star et al., 2012). Results from this study confirmed that IAA and nitric oxide produced by *A. brasilense* also play an important role in co-inoculation of legumes with rhizobia and azospirilla, and confirmed that IAA production by *A. brasilense* is a key component for enhancement of secretion of nod-gene-inducing flavonoids by legume roots.

3. Available genomes of *Azospirillum* sp.

To date, four whole azospirilla genomes have been published: *A. brasilense* Sp245 (Wisniewski-Dyé et al., 2011), *A. brasilense* Az39 (Rivera Botia et al., 2014), *A. lipoferum* 4B (Wisniewski-Dyé et al., 2011) and *Azospirillum* sp. B510 (Kaneko et al., 2010), while four more genomes were sequenced but the information is still unpublished (*A. brasilense* Sp7; *A. brasilense* FP2, *A. brasilense* CBG497 and *A. thiophilum* BV-S). *A. brasilense* Sp245 is one of the most studied strains world-wide and is considered as a type strain for this species. It was isolated from surface-sterilized wheat (*Triticum aestivum* L.) roots in Paraná state from the South region of Brazil (Baldani et al., 1986) and was one of the most promising strains for wheat inoculation in Brazil during the 80s. *A. lipoferum* 4B was isolated from the rhizosphere of rice (*Oryza sativa* L.) (Bally et al., 1983) and it was successfully used as inoculant to increase rice yield under field conditions (Charyulu et al., 1985). *Azospirillum* sp. B510 is an endophytic bacterium isolated from surface-sterilized stems of rice plants in Kashimadai, Japan (Elbeltagy et al., 2001). *A. brasilense* strain CBG497 was isolated from the rhizosphere of maize (*Zea mays* L.) plants grown on soils with pH of 8.0 from the North-East region of Mexico (Nelson and Knowles, 1978). *A. brasilense* Sp7 (ATCC 29145) was isolated by Joana Döbereiner from pangola grass (*Digitaria decumbens*) plants from Rio de Janeiro in Brazil and proposed by Tarrand et al. (1978) as the type strain for the specie. Finally, *A. brasilense* FP2 is a spontaneous mutant of Sp7 (Nal^r and Sm^r) obtained by Pedrosa and Yates (1984).

3.1. *Genomic analysis of Azospirillum brasilense Az39*

A. brasilense Az39 was isolated in 1982 from surface-sterilized wheat seedlings in Marcos Juarez (Córdoba, Argentina) and selected for inoculant formulation based on its ability to increase crop yield of maize and wheat under agronomic conditions (Díaz-Zorita and Fernández-Canigia, 2009). The mechanisms responsible for the growth promotion by the strain Az39 have been partially unraveled (Perrig et al., 2007; Rodriguez Cáceres et al., 2008). The genome of *A. brasilense* Az39 is divided in six replicons (one chromosome, three chromids and two plasmids) and consists of 6311 protein-coding sequences: 2763 on the chromosome, 1605 on AbAZ39_p1, 744 on AbAZ39_p2, 534 on AbAZ39_p3, 557 on AbAZ39_p4, and 108 on AbAZ39_p5. The putative genes involved in plant growth promotion have been related to the following mechanisms: nitrogen fixation; auxin, cytokinin, gibberellin, ethylene, abscisic acid, polyamines and nitric oxide biosynthesis; biofilm formation and type I, II and VI secretion systems. Identical mechanisms and similar genes encoding for homologs proteins were previously identified in *A. brasilense* Sp245 genome (Wisniewski-Dyé et al., 2011) and later in Sp7, FP2 and CBG497 genomes (unpublished). Contrarily, in the case of *A. lipoferum* 4B, *Azospirillum* B510 (Wisniewski-Dyé et al., 2011) and *N. amazonense* Y2 (Sant'Anna et al., 2011) their genome sequences reveals differences at level of *quorum sensing* and IAA biosynthesis, among other mechanisms. In the case of *quorum sensing*, the presence of genes encoding for LuxI homologs proteins was not detected in all sequenced strains of *A. brasilense*. For IAA biosynthesis, no evidence has been found for the existence of *ipdC* or aldehyde dehydrogenase genes in the genome sequence of *A. lipoferum* 4B. In the case of *Azospirillum* sp. B510, the genome sequence revealed two candidate genes proposed to be involved in the IAM pathway by Kaneko et al. (2010). However, we question their role in IAA biosynthesis due to low similarity with known *iaaM* and *iaaH* genes and the genome sequence of *A. amazonense* could not reveal the presence of genes involved in the IPyA or IAM pathway (*ipdC, iaaM or iaaH*), but revealed a gene encoding a protein with about 70% similarity to nitrilases of *A. thaliana*.

Considering that *A. brasilense* Az39 is the only strain successfully used for agriculture during the last 50 years in diverse environments of Argentina and others in South America, with a full ungapless sequence of their genome, should be consider as a useful tool for molecular studies and an excellent basis for in-depth comparative genome analyses, to understand the specific mechanisms of *Azospirillum*-plant interactions.

4. The sustainable use of *Azospirillum* spp.

4.1. *Azospirillum-based inoculants and plant growth promotion*

Due to the positive effects of *Azospirillum* sp. on growth of several plant species, this genus has attracted the attention of researchers to develop specific strains as inoculant for use in agriculture. Most inoculants claim plant growth promoting effects under suboptimal conditions for growth, such as limited plant available nitrogen, resulting in a higher final yield compared to non-inoculated treatments. However, in many field experiments around the world, inoculation studies have not been reproducible, therefore questioning the capacity of these inoculants. In South America, a flourishing inoculant business is being developed, possibly due to the large surface dedicated to extensive agriculture and the reproducible results under field conditions. The rest of this section focuses on Argentina as a case study of *Azospirillum*-based inoculants in South America and other parts of the world. From 1981 to 1996, the Microbiology and Zoology Institute for Agriculture (IMYZA) from the National Institute for Agricultural Technologies (Instituto Nacional de Tecnología Agropecuaria, INTA) based on Castelar (Buenos Aires, Argentina), developed an intensive program with the main objective to select and to identify *Azospirillum* sp. strains and to evaluate their ability to promote plant growth in different crop species. The experiments showed a more pronounced effect with *A. brasilense* as compared to *A. lipoferum* on most of the evaluated plant species, and allowed selection of *A. brasilense* Az39 as the most promising strain for inoculant formulation, based on its ability to increase growth and grain yield of evaluated crops in the range of 13–33%. Based on this information, the National Service of Agricultural Health (Servicio Nacional de Sanidad Agropecuaria, SENASA) made a nationwide recommendation of the native strain *A. brasilense* Az39 for inoculant production for maize, wheat and other non-legume species. From a physiological point of view, the plant growth promoting capacity of *A. brasilense* Az39 has been confirmed due to its effectiveness in increasing the productivity of inoculated crops in large number of assays under field conditions, during the last 30 years (Díaz-Zorita and Fernández-Canigia, 2009). *A. brasilense* Az39 inoculated alone or in combination with *Bradyrhizobium japonicum* E109 has the capacity to promote seed germination and early growth in soybean [*Glycine max* (L.) Merrill], wheat and maize. This strain is able to produce and releasing Gibberellic Acid (GA_3), zeatin (Z) and also IAA in culture medium (Perrig et al., 2007) at concentration that produce morphological and physiological changes in treated seeds or seedling of several plant species (Cassán et al., 2009a). *A. brasilense* Az39 possesses the capacity to promote germination and early growth in a phytohormone-concentration depending way. Bacterial releasing of GA_3, Z and IAA into culture medium during fermentation showed differences during exponential or stationary growth phase (Cassán et al., 2010). In stationary growth phase Az39 accumulates more phytohormones than in exponential growth phase and this accumulation alters the "inoculant" capacity to promote the germination and early plant growth. This action has been defined as the "hormonal effect of inoculation" by Cassán et al. (2010) and could be extended to several plant species and phytohormone-producing microorganisms.

4.2. Field crops responses to the inoculation with Azospirillum sp.

The crops usually show growth reduction with different environmental stress conditions that frequently occur simultaneously and it is recognized that *Azospirillum* sp., among other microbes, ameliorate these conditions and promote better growth and productivity. The plant's response to abiotic stresses involves the activation of the hormones, redox, nitric oxide, kinase, and calcium signaling pathways (Gassman and Appel, 2016), and most of these paths are also triggered by diverse natural soil bacteria. The interplay between the various signaling pathways in response to abiotic stressors is complex, especially in the field, where the plant is exposed to a combination of stressors. The contribution of soil microorganisms on plant productivity is often studied under isolated or controlled growth conditions, providing information regarding the promising contributions of their use as inoculants and enriching the rhizosphere. But, this approach has limitations for its extrapolation to field production conditions.

Studies performed in large agricultural settings under regular environmental and crop management practices are scarce and limited to a few regions. Díaz-Zorita et al. (2015), reviewed 47 articles worldwide published during the last decade describing 347 cases of grain crop production responses to the application of diverse *Azospirillum* sp. inoculants in 12 countries. They observed that most of the studies were performed in cereals crops (86.7%), mainly dryland maize. Most experiments were located in Brazil and in different countries of Asia. Among all the reported crops, the mean grain response to the inoculation with *Azospirillum* sp was 10.0%, with greater yields in winter cereals (14.0%) rather than in summer cereals (9.5%) or legumes (6.6%). The positive responses to the inoculation with *Azospirillum* spp. were also reported by Bashan et al. (1989), working with non-cereals crops, and reviewed by Okon and Labandera-Gonzalez (1994), on many other crops, including both row crops and vegetables grown in diverse environments and production conditions.

There is a common agreement among most of these large studies about the inconsistency of the responses to inoculation. In general, the production response to the inoculation with *Azospirillum* sp. is successful in 70% of the experiments (Okon and Labandera-Gonzalez, 1994; Díaz-Zorita and Fernández-Canigia, 2009). The lack of consistency in the results from field experiments is one of the main obstacles impeding a widespread commercial use of plant growth promoting microorganisms like *Azospirillum* sp. (Dobbelaere et al., 2001). The interpretation and application of these results are variable and is inconclusive, in part, because of the complex interaction between the different modes of action of *Azospirillum* sp. on plants, and also due to the multiplicity in abiotic stress conditions to be mitigated in presence of the microorganisms. Thus, we support the theory of multiple mechanisms acting in sequential or cumulative patterns (Bashan and de-Bashan, 2010). In addition, the methods of inoculation as well as the diverse crop management practices strongly related with the occurrence of abiotic limitations for crop growth (i.e. irrigation, fertilization, genotypes, combination with other beneficial microbes, etc.) could partially explain the inconsistent results in field experiments.

4.3. Carriers and modes of application under field conditions

Azospirillum sp., among other microbes with recognized beneficial effects on plant growth, has been formulated in inoculants, also named biofertilizers. Generally, the microbes introduced into an inoculant formulation are representative of the bacterial population that is already present in the soil. The inoculation increases the numbers of cells in the rhizosphere, ensuring an early colonization and increasing the probability that the beneficial effects of the selected strains can be exerted on plants (Balandreau, 2002).

The inoculation with *Azospirillum* sp. in field crops is generally done treating the seeds with liquid or solid carriers containing the microorganisms or applying them directly in the sowing furrow during seed sowing. In rice and other intensive grown crops, transplanting seedlings soaked in a solution containing *Azospirillum* sp. has been also described in the bibliography to deliver the microbes to the rhizosphere (Govindarajan et al., 2008; Islam et al., 2012; Khalid et al., 2011). Foliar and soil sprayed application formulations containing these bacteria were also described in several studies, but with controversial results and not clear recommendations about its management (i.e. growth stage at the moment of application, concentration of living cells delivered per plant, environmental and application conditions for its adequate use, etc.) (El Habbasha et al., 2013; Fukami et al., 2016).

Currently, the seed treatment at the moment of sowing is the preferred mode of inoculation with *Azospirillum* sp., mainly done without the application in slurry with other products (i.e. micronutrients, fungicides, insecticides, polymers, etc.). Most of the reports discussed in this chapter describes the effect of seed treatments applied at the moment of sowing with liquid or peat based formulations containing different strains of *Azospirillum* sp. Several other formulations containing *Azospirillum* sp. have been developed for treating the seeds long-term before sowing. One of these allows to include the microorganism in the formulation of alfalfa (*Medicago sativa*) coating treatments also in combination with other chemical compounds providing a long term survival of azospirila (Díaz-Zorita et al., 2012a). The risk of incompatibility between the microorganisms present in the inoculants and the diverse compounds (i.e. fungicides, insecticides, micronutrients, pigments, etc.) used in seed treatments limits the beneficial contribution of inoculation, and needs to be investigated. Thus, alternative methods for seed inoculation as well as the integral management of the seed treatment process are often developed. The studies based on the application of the inoculants in the furrow of sowing are scarce and still under discussion but there is no doubt that it is a recommended option for reducing the risk of low compatibility or viability of the microbe with other treatments applied to the seeds (Hungria et al., 2013). Fukami et al. (2016) compared diverse methods of inoculation (i.e. in-furrow, soil spray at sowing and foliar spray after seedling emergence) and identified effective alternative methods of inoculation (Fig. 2). Foliar spray improved the colonization of leaves while the soil inoculation favored roots and rhizosphere colonization. Authors reported positive effects of inoculation on the volume of maize roots and plant height when the inoculant was sprayed in the soil at the emergence of the seedlings. But, more wheat tillers per plant were described when the inoculant was sprayed on the

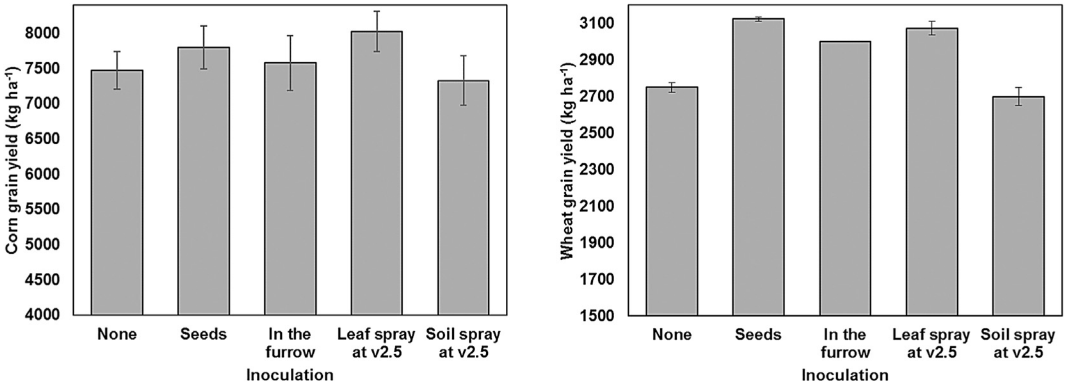

Fig. 2. Grain yield of crops inoculated with *A. brasilense* under different systems of inoculation. Mean of two corn sites and one wheat site each under two levels of N fertilization. The vertical bars represent the standard error of the means (Adapted from Fukami et al., 2016).

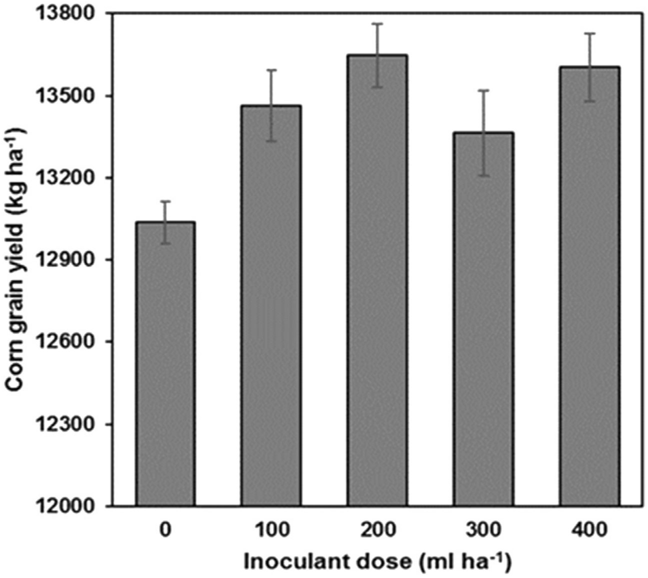

Fig. 3. Corn Grain yield of crops inoculated with *A. brasilense* in the furrow of plating. Mean of three nitrogen fertilization levels. The vertical bars represent the standard error of the means (Adapted from de Morais et al., 2016).

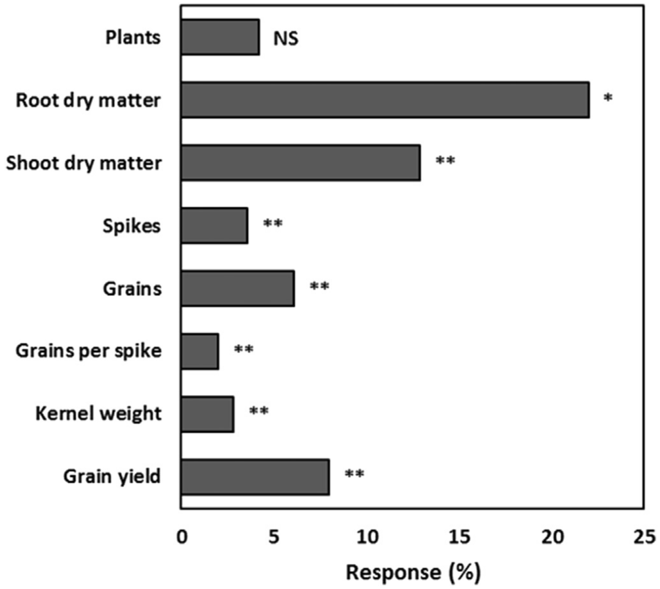

Fig. 4. Increase of grain yield parameters and production of *A. brasilense* inoculated wheat crops in the pampas, Argentina. Mean of 297 experimental sites. Statistics from LSD (T): NS = non-significant differences ($p > 0.10$), * = $p < 0.05$; ** = $p < 0.01$ (Adapted from Díaz-Zorita and Fernández-Canigia, 2009).

Table 2
Increase of wheat and barley grain yield parameters due to inoculation with *A. brasilense* Sp246. Values represent the percentage of increase compared with non-inoculated plants. Mean of three N fertilization levels and two growing seasons (Adapted from Ozturk et al., 2003).

	Wheat	Barley
	%	
Spikes m^{-2}	7.2	6.6
Grains $spike^{-1}$	5.9	8.1
Grains m^{-2}	13.1	14.7
Grain yield m^{-2}	14.7	17.5
Protein content	4.1	5.1

leaves.

de Morais et al. (2016), in field trials performed in the cerrado region of Brazil, showed positive grain yield responses of maize crops when inoculated in the furrow of planting at a dose greater than 200 ml ha^{-1} (Fig. 3). These promising results support that other than seed treatments could be alternative methods for the inoculation of crops and soils with *Azospirillum* sp. but still requires several practical adjustments (i.e. dose of inoculant per plant, total volume of application for foliar or soil spraying, etc.).

The excessive manipulation of seeds is discouraged because desiccation, high temperatures, ultraviolet light, or toxic compounds may affect microbial survival (Díaz-Zorita et al., 2015). Also, sowing as well as the furrow treatment application should be done in a moist seedbed, avoiding the seeds to be exposed to desiccation or direct sun light.

4.4. Changes in yield components in inoculated crops

Azospirillum sp. inoculation promotes maize productivity under different environmental conditions, usually correlated with the increase of the root surface that leads to an increase in the soil volume exploration. In grasses, the inoculation with *Azospirillum* sp. alters the morphology of the roots increasing the number of

Table 3
Range of wheat and corn grain yield increase in response to the inoculation with *A. brasilense* in soils from the Buenos Aires province, Argentina, compared to non-inoculated experiments (Adapted from García de Salomone, 2012).

Soil type	Wheat	Corn
Humic Hapludoll	2–34%	6–77%
Typic Argiudoll	0–30%	0–7%
Entic Hapludoll	2–32%	3–13%
Acuic Argiudoll	0–10%	–
Aeric Argiudoll	–	2–94%
Vertic Argiudoll	–	5%

lateral roots and root hairs (Steenhoudt and Vanderleyden, 2000). More early plant height growth has been frequently reported in *Azospirillum* sp. inoculated crops, however the relative contribution in above ground growth is smaller than in root growth and generally decreased with the development of the crops (Dobbelaere et al., 2001; Naiman et al., 2009). Veresoglou and Menexes (2010), based on the aggregated analysis of multiple published articles, described that wheat grain yield responses to inoculation represented approximately 50% of the aboveground dry weight responses. Maize crops treated with *A. brasilense* and grown under dryland conditions, among different regions in South America, showed a greater vegetative growth with a greater shoot dry matter accumulation (7.9%) and also a greater number of grains at harvest (4.3%). But, no significant differences on single grain weight were described between treatments (Díaz-Zorita et al., 2012b). Similar responses have been described in the case of wheat and barley (*Hordeum vulgare* L.) crops under diverse environmental conditions. A positive relationship in the relative contribution of the inoculation with *Azospirillum* sp. between the aboveground growth and the grain yield of wheat crops was described from the meta-analysis of 59 worldwide published articles (Veresoglou and Menexes, 2010). The reported benefits in crop grain yield are mostly explained for the increase in the quantity of grain produced in response to the better vegetative growth in inoculated crops with minimal effects on yield components determined during the seed filling period like single grain weight or its composition (Table 2, Fig. 4).

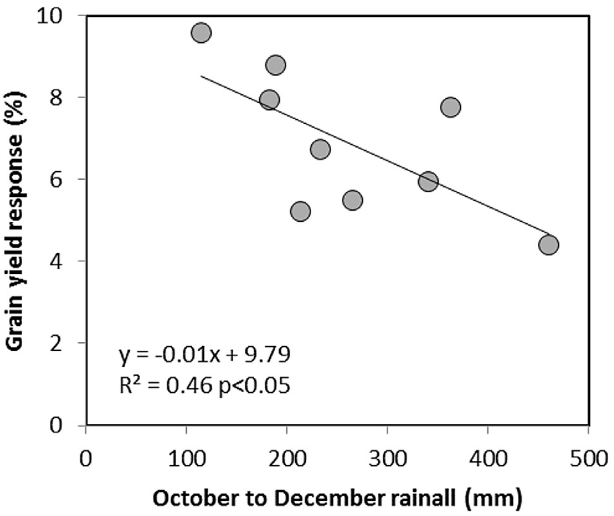

Fig. 5. Mean relative seasonal grain yield responses to the application of *A. brasilense* on corn crops of the pampas region (Argentina) and rainfalls during vegetative (October to December) growing stages (Adapted from Díaz-Zorita, 2012).

4.5. Field characteristics and crop responses to inoculation

The inoculation of crops with *A. brasilense* has showed the enhancement in plant growth and productivity in diverse environments. But, as crop productivity and grain yield are strongly affected by the environment, the success of the practice lies on both the inoculation practice and environmental conditions. Unfortunately, most field studies have been performed in single locations or during few production seasons, and the interpretation of the contribution of *Azospirillum* sp. under random temporal and spatial conditions that currently affects crop production is limited. Only, large agricultural studies performed during multiple growing seasons and experimental sites provide valuable information.

The responses to the inoculation with *Azospirillum* sp. vary among soil types and crops (Table 3). It has been proposed that crop stressful conditions like short term droughts, mainly during early growth stages of the inoculated crops, among others, severely affects the success of the inoculation. It has been described that the wheat grain response to the inoculation with *A. brasilense* in the semiarid and subhumid pampas region of Argentina decreases when the rainfalls increase relative to the environmental water demand (measured as evapotranspiration) as well as when the soil organic matter content increase (Rodriguez Cáceres et al., 2008).

Díaz-Zorita et al. (2012b), based on 298 experimental trials from Argentina, Bolivia, Brazil, Paraguay and Uruguay, also concluded that maize responses to the inoculation were greater under stressful conditions during the early growth of the crops or in the absence of nitrogen fertilization. When rainfall is scarce during the early stages of growth of the crops, the positive effect of the inoculation practice on crop yields is evident (Fig. 5). Under well-watered conditions, the changes in root development, attributed to *Azospirillum* sp. inoculation, are less evident. During early abiotic stress conditions, *Azospirillum* sp. increases root length, surface and volume compared with untreated crops (Dobbelaere et al., 1999; Kapulnik et al., 1985). But, under strong stressful growing conditions (i.e. severe droughts, major nutrients limitations, etc.) the responses to the inoculation treatment are rarely observed (Lana et al., 2012; Mehnaz et al., 2010; Naiman et al., 2009; Naseri et al., 2013).

The contribution of the environmental factors that affects the growth of the crops and the inoculation practice response normally occurs at random. Under the complexity of interacting random factors, a better interpretation of the results requires the analysis based on the frequency of their occurrence as well as the discrimination among hierarchical productivity factors. For conclusive observations regarding the crop responses to the inoculation with *Azospirillum* sp., a large number of observations are needed to describe significant differences between treatments. The analysis of data from 432 wheat and 225 maize rainfed field trials in Argentina showed similar frequency of responsive sites and in the mean relative responses among crops (Fig. 6). Thus, the analysis of distribution response values is a more realistic approach to measure the crop performance (Díaz-Zorita et al., 2015). For example, based on 316 field experiments, maize yields yielded from 2.02 t ha^{-1} to 18.65 t ha^{-1}. Although percentile values differed along the whole distribution, only mean yields of the treatments distribution in the range from 5 to 95% were significant (Table 4). The probability to find differences in both extremes of the data distribution was low; however, in the central 90% of the distribution, in crops yielding between 6.00 and 12.00 t ha^{-1}, crops inoculated with *A. brasilense* had a consistent higher probability of greater yields than control crops (Table 4).

The meta-analytical approach also contributes to quantify the

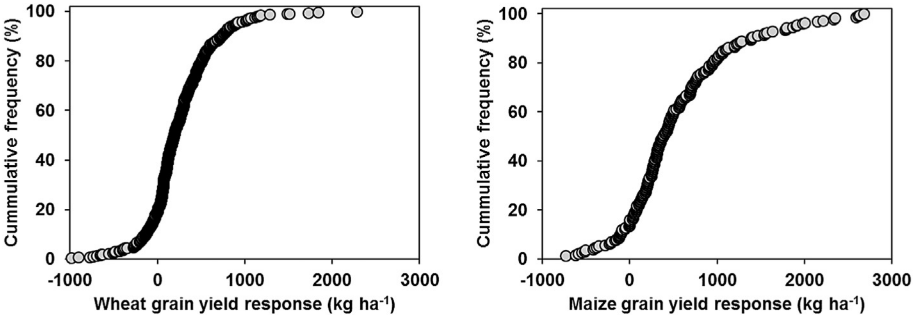

Fig. 6. Cumulative frequency distribution of wheat and maize grain yield responses to the inoculation with *Azospirillum brasilense* under rainfed production conditions in Argentina (Adapted from Rubio and Díaz-Zorita, 2015).

contribution of *Azospirillum* spp. inoculation on crop growth and grain yields. Veresoglou and Menexes (2010) performed a meta-analysis of aggregated data of wheat growth in the presence of *Azospirillum* spp. from 59 published articles under both pot or field conditions. Based on the analysis of 480 trials, the authors validated the benefits of wheat inoculation with *Azospirillum* spp. and identified key determinants for the variable crop growth responses. The inoculation response varied depending on the amount of N fertilization (maximum in the absence of N fertilization), the identity of wheat cultivars (*Triticum aestivum* superior to *Triticum durum*) and the *Azospirillum* isolate (*Azospirillum lipoferum* more effective than *Azospirillum brasilense*).

4.6. Inoculation responses and crop management practices

Crop management practices influence the growth of crops, including the performance of *Azospirillum* sp. inoculated crops in the presence of abiotic stresses (i.e. nutrient deficiencies, water stress, etc.). The specificity between *Azospirillum* strains and plant species and genotypes is not clearly evident, thus the selection of strains under specific environmental and production (i.e. crops, management practices, etc.) conditions is a key factor for achieving a successful plant response to inoculation (Hungria, 2011). For example, the response of rice to *Azospirillum* sp. inoculation is variable and depends on the interaction between plant cultivars and *Azospirillum* species and strains (Okumura et al., 2013). For example, a plant genotype-*Azospirillum* sp. has been described in the North part of the maize production region of Argentina (García de Salamone and Dobereiner, 1996). But, under regular production conditions, where multiple interactive factors are involved in the response to the inoculation treatment, the type of germplasm in wheat crops seemed to have a minor effect on yield (Fig. 7). From the same integral study, among 297 experimental sites located in the pampas region of Argentina, the wheat response to the inoculation with *Azospirillum* sp. showed differences depending on the previous cultivated crop and the fertilization practice.

Table 4
Mean values of percentile ranges in the empirical distribution of corn grain yields from control and *A. brasilense* inoculated crops in a database from 316 field trials performed in the pampas region (Argentina) under regular production practices. P = statistical significance, n = number of sites considered in each range, SE = standard errors of the means, and ns = non-significant differences between means (Adapted from Díaz-Zorita et al., 2015).

Treatment	Range of the empirical distribution				
	0–5	5–25	25–75	75–95	95–100
	Grain yield (kg ha^{-1})				
Control	3691	6118	8755	11,541	13,838
Inoculated	3912	6566	9216	11,948	14,448
P	ns	<0.001	<0.001	<0.001	ns
n	16	63	157	64	16
SE	209	92	78	82	316

Strongest yields responses to *Azospirillum* sp. inoculation of wheat crops were obtained by crop rotation with sunflower (*Helianthus annus* L.) or maize. These results suggest that the site condition related to the crop in rotation with wheat is a limiting factor for the growth of the crop and its response to inoculation. The sunflower production area in Argentina mostly lies within the semiarid pampas region, with frequent water stress conditions that limit wheat production. Also, it is well known that the residues from maize crops have a negative effect in wheat establishment, reducing the stands of plants and its productivity. In both situations, wheat production in rotation with maize or with sunflower, the inoculation with *Azospirillum* sp. provides beneficial conditions for root growth, enlarging the soil volume exploration and providing more resources for the formation of tillers. In the other hand, the response of wheat to the inoculation was not significant in areas where pastures were frequently grown probably because better soil fertility conditions related with large soil organic matter contents were found (Díaz-Zorita and Fernández-Canigia, 2009).

Veresoglou and Menexes (2010), among diverse environmental conditions, also reported positive responses in the growth of wheat plants inoculated with *Azospirillum* sp. They concluded that the benefit varied depending on the nitrogen fertilization rate, the genotype of wheat and also the strain of *Azospirillum* sp. applied. The fertilization effect on the response to the inoculation is controversial and it varies depending on the applied nutrient, the rate as well as the crop response to this practice itself. It has been described that nitrogen fertilization is beneficial to wheat crops responses to *Azospirillum* sp. inoculation because of the promotion in nitrogen uptake and use (Saubidet et al., 2002). But, the grain yield parameters of wheat and barley crops inoculated with *A. brasilense* diminished when the rate of nitrogen fertilization increased. Furthermore, under relatively high nitrogen availability the plant response to *Azospirillum* sp. inoculation was not observed (Ozturk et al., 2003). In sites where nitrogen availability is a limiting factor for the normal productivity of cereals the plant response to the inoculation is frequently observed. In a study performed in two representative sites in the semiarid-subhumid pampas cultivated with rye (*Secale cereale* L.) as a cover crop, it was observed that the mean response in dry matter production of *A. brasilense* inoculated

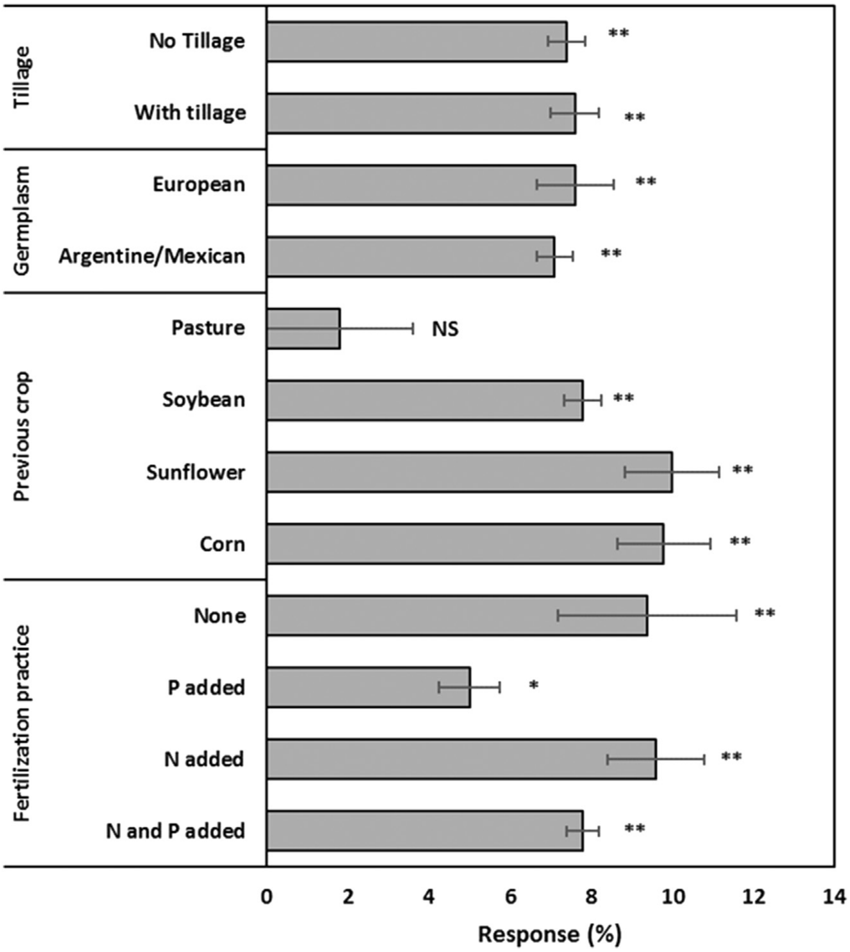

Fig. 7. Wheat grain yield response to crop and soil management practices, subjected to seed inoculation with a liquid formulation containing *A. brasilense* across 297 experimental sites in the pampas region of Argentina. Horizontal bars represent the standard error of the means. Statistics from LSD (T): NS = non-significant differences ($p > 0.10$), * = $p < 0.05$; ** = $p < 0.01$ (Adapted from Díaz-Zorita and Fernández-Canigia, 2009).

Table 5
Shoot dry matter production of rye cover crops inoculated with *A. brasilense*. Parameters were measured after 150 days of growth in two sites (A and B) from the semiarid-subhumid pampas (Adapted from Cristian Alvarez, pers.com.).

Seed inoculation	N fertilization (kg ha^{-1})	Sites		
		A	B	Mean
		Dry matter (kg ha^{-1})		
None	0	4270	2893	**3961**
	40	5087	3593	
A. brasilense	0	5227	3348	**4495**
	40	5775	3628	

plants was 0.5 t ha^{-1}, equivalent to 13% of increase (Table 5). This response was mainly observed when nitrogen fertilization was performed and suggests the complementary need of both practices (inoculation and fertilization) for achieving fast and abundant aboveground biomass.

The response of the crops to the inoculation under severe availability of nutrients is also limited or negligible. For example, from the evaluation of 10 wheat production sites located in the pampas region with limitations in the soil extractable phosphorus concentrations, it was observed that the grain yield response to the seed inoculation with *A. brasilense* was only significant in treatments with phosphorus fertilization (Fig. 8). In these sites, the mean response to the fertilization was almost 55% greater in the inoculated crops (0.81 t ha^{-1}) than in the absence of this practice (0.52 t ha^{-1}). The mean response to the inoculation in the fertilized crops was 0.29 t ha^{-1}, equivalent to the increment in 6% the grain yields compared with the control without the use of the biological seed treatment.

The combined inoculation of legumes with rhizobia and azospirilla, among other beneficial soil microorganisms, because of their complementary biological activities in plants, could improve the performance of the plants. Although the contribution of the co-inoculation with rhizobia and azospirilla on the productivity of diverse legume crops and pastures is promising, the available information under large production conditions is limited. The results from 21 field trials with alfalfa pastures performed in the pampas region of Argentina showed that the application of biological seed treatments combining *Sinorhizobium meliloti* and *A. brasilense* enhanced both the establishment of plants and the forage production (Díaz-Zorita et al., 2012a). The inoculation with *S. meliloti* showed 14.7% more dry matter production than the control without biological seed treatment. When *A. brasilense* was combined in the biological treatment, the dry matter response was doubled (28.5%). Between both biological seed treatments, the dry matter production per plant after 150 days of growth was greater when *A. brasilense* was combined with *Sinorhizobium meliloti* (1.69 kg plant^{-1}) than when only *S. meliloti* was applied (1.65 kg

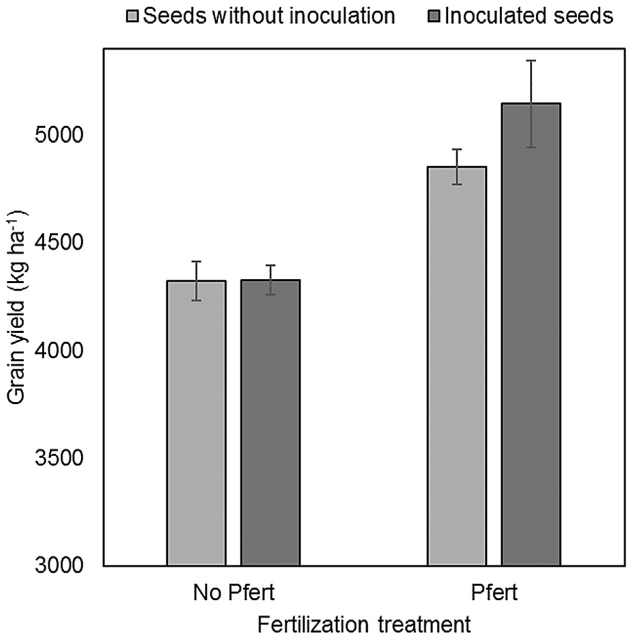

Fig. 8. Wheat grain yield response seed inoculation with *A. brasilense* and phosphorus fertilization (Pfert) in the pampas region of Argentina. Mean of 10 sites performed during the 2015/16 growing season. Vertical bars represent the standard error of the means (Adapted from Díaz-Zorita unpublished data).

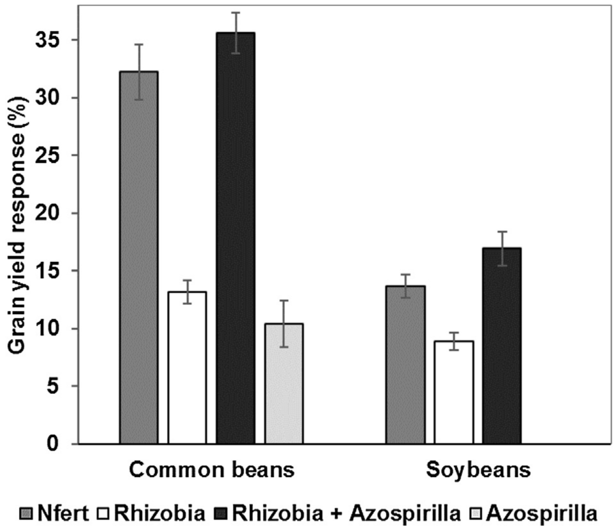

Fig. 9. Grain yield response of common beans and soybeans crops to rhizobia and azospirilla inoculation. Mean of four sites from Brazil. Nfert = nitrogen fertilization, 80 kg of N ha^{-1} in common beans and 200 kg ha^{-1} in soybeans. The vertical bars represent the standard error of the means (Adapted from Hungria et al., 2013).

plant^{-1}). Hungria et al. (2013) also showed more grain yields of soybean and common bean (*Phaseolus vulgaris*) when combining rhizobia seed inoculation with in-furrow application of *A. brasilense* in four sites from Brazil (Fig. 9).

5. Registration and production of inoculants with *Azospirillum* sp. in South America

In the South cone of South America, there are 104 biological products containing *Azospirillum* sp. available for commercialization in the region. The inoculants done for more than 50 companies and mainly formulated in liquid carriers (Tables 6–9). Most of them are produced in Argentina (85 products) and only 16 in Brazil, 2 in Uruguay and 1 in Paraguay. All the available products for commercialization in the Argentine market are produced in Argentina (Table 6). But in Brazil (Table 7) and Uruguay (Table 8), the products are both locally done or imported from Argentina. In Paraguay (Table 9) the production is locally produced and also imported from both Argentina and Brazil.

The Az39 strain of *Azospirillum brasilense*, is the active principle in 65% (68 products) of these inoculants and only in only 6 products it is combined with other strains (CFN535) of *Azospirillum brasilense* (1 product) or with *Pseudomonas fluorescens* (5 products). The combination of the strains Abv5 and Abv6 of *Azospirillum brasilense* is used for the formulation of 11 products and the combination of strains Az78 and Az70 of *Azospirillum brasilense* is in 2 products. The rest of azospirilla inoculants is formulated with single strains (Abv5, AzM3, AzT5, 1003, Tuc 27/85, Tuc 10/1 and 11005). There are no other species of *Azospirillum* used for the formulation of azospirilla containing inoculants in the region. Liquid carriers are the more used for the formulation of these biological products (82%) while 8% of them are formulated on solid carriers like peat or bentonite. The more frequent shelf life of the registered products is 6 months (data not shown). Only one product shows long life survival of the azospirilla life and recommended for industrial seed treatments (i.e. pelletized alfalfa).

Although 13 crops have been recommended for the use of these biological products, the registration is mainly for maize (73 products) and wheat (64 products) crops. Sorghum (*Sorghum bicolor*) (29), sunflower (13), soybean (7), grasses and winter cereals for grazing (4), rice (3), barley (3), cotton (*Gossypium hirsutum*) (3), oats (*Avena sativa*) (2), sugar cane (*Saccharum officinarum*) (1), tobacco (*Nicotiana tabacum*) (1) and lettuce (*Lactuca sativa*) (1) are the rest of the crops recommend for the application of commercial products containing *Azospirillum brasilense*.

In Brazil, most of the commercialized products were allocated in the maize grain production market. Based on 2014 data, approximately 2.9 million doses of azospirilla inoculants were commercialized covering almost 2.8 million ha. Almost 2000 ha cultivated with maize have been treated with azospirilla inoculants during the 2014 growing season in Uruguay. In the other hand, in Argentina these products are more used for the production of wheat crops and also in combination with rhizobia inoculants for soybean production covering approximately 300,000 ha.

6. Concluding remarks

Azospirillum sp., is probably the most studied genus of associative plant growth promoting rhizobacteria due its capacity to colonize many plant species. Multiple and complex mechanisms have been described in the microbe-plant interaction (i.e. nitrogen fixation and phytohormones biosynthesis); but, none of them individually has been identified to support the changes in plant growth. Currently is agreed that the combination or the sum of them operate providing variable benefits to the growth of the plants interacting with also diverse stressful environmental conditions. During the last two decades, the inoculation of selected *Azospirillum* sp. strains on diverse crops under field conditions has been intensively studied. It has widely shown their consistent benefits improving plant growth, development and productivity under controlled and field conditions. The use of azospirilla inoculants for crop production is a promising and increasing practice mostly in crop management conditions (i.e. dryland, limited fertilization, etc.), where the occurrence of abiotic growth stresses is frequent. The benefits of its use complements the application of other recommended, but still limited, production practices like

Table 6
Biological products formulated with *Azospirillum* sp. registered in Argentina at 2015. Source: SENASA and INTA (unpublished data). References: C: corn; W: wheat; S: sorghum; Soy: soybean; Sun: sunflower; O: oats; B: barley; Wg: winter grasses; G: grasses in pastures; Cot: cotton; L: lettuce; Sug: sugarcane; T: Tobacco, Clo: clover. Combined products with other PGPR are referred as comb.

Origin	Brand	Active principle	Company	Carrier	Crop
Argentina	Axion Plus	*A. brasilense* Az39	Agro Franquicias SA	Liquid	W
Argentina	Full Bacter	*A. brasilense* Az39	Agro Invest SRL	Liquid	W, C
Argentina	Alter Promaz Tls	*A. brasilense* Az39	Alterbio SA	Liquid	W, B, O
Argentina	Facyt Az	*A. brasilense* Az39	Ayui SRL	Liquid	W, C, Sun, B, O
Argentina	Nitrafix	*A. brasilense* 11005	BASF Argentina SA	Liquid	W, C, S, Soy
Argentina	Nitro Fix Az	*A. brasilense* Az39	Bilab SA	Liquid	W, C, Sun, Cot
Argentina	Axion Plus Az	*A. brasilense* Az39	Bilab SA	Liquid	C
Argentina	Axion Plus Az	*A. brasilense* Az78 + Az79	Bilab SA	Liquid	W, C
Argentina	Hober Soy	*A. brasilense* Az78 + Az79	Bilab SA	Liquid	Soy
Argentina	Azomix	*A. brasilense* Az39	Bionet SRL	Liquid	W
Argentina	Azomax	*A. brasilense* Az39	Campomax SH	Liquid	W
Argentina	Beterseed *Azospirillum*	*A. brasilense* Az39	Cao Rocio	Liquid	W, C
Argentina	Grow Azp	*A. brasilense* Az39	Ceres Demeter SRL	Liquid	W, C
Argentina	Azosnitro	*A. brasilense* Az39	Cergen SRL	Liquid	W, C
Argentina	Gramibac	*A. brasilense* Az39	Chemical-Bio SA	Liquid	W
Argentina	Radixius	*A. brasilense* Az39	Chemical-Bio SA	Liquid	C
Argentina	Crinigan Maíz	*A. brasilense* Az39	Crinigan SA	Peat	C
Argentina	Ray Green	*A. brasilense* Az39	Ecofertil SRL	Peat	Soy
Argentina	Ray Green	*A. brasilense* 1003	Ecofertil SRL	Liquid	W, C, Sun
Argentina	G3 Azubac	*A. brasilense* Az39	Emfag SA	Liquid	W
Argentina	Sower	*A. brasilense* Az38	Farmchem SA	Liquid	W
Argentina	Azos B	*A. brasilense* Az39	Fitogenia SRL	Liquid	W, C
Argentina	Forte	*A. brasilense* Az39	Fitoquimica SA	Liquid	W, C
Argentina	Azp 2000	*A. brasilense* Az39	FPC SA	Liquid	W, C, B
Argentina	Trigalazo	*A. brasilense* Az39	Fragaria SRL	Liquid	W
Argentina	Graminazo Plus	*A. brasilense* Az39	Fragaria SRL	Liquid	W, C, S
Argentina	Cazo	*A. brasilense* Az39	Fragaria SRL	Liquid	C
Argentina	FosW N	*A. brasilense* Az39	Green Quality SA	Liquid	W, C, S
Argentina	Graminante Maíz	*A. brasilense* AzM3	Lab. Alquimia SA	Liquid	C
Argentina	Graminante Trigo	*A. brasilense* AzT5	Lab. Alquimia SA	Liquid	W
Argentina	N Azospirrillum	*A. brasilense* V5 + V6	Laboratorio Biotech SA	Liquid	W, Sun, Sug, S, Cot
Argentina	BioNitrosem Azo	*A. brasilense* Az39	Laboratorio Biotech SA	Liquid	W, C, Sun
Argentina	Azollum H	*A. brasilense* Tuc 27/85	Laboratorio San Pablo SRL	Liquid	W
Argentina	Azollum	*A. brasilense* Az39	Laboratorio San Pablo SRL	Liquid	W
Argentina	Azollum Maíz	*A. brasilense* Tuc 27/85	Laboratorio San Pablo SRL	Liquid	W, C
Argentina	Tabazoll Duo Plus	*A. brasilense* Az39	Laboratorio San Pablo SRL	Liquid	T
Argentina	Macromix	*A. brasilense* Tuc 10/1	Laboratorio San Pablo SRL	Liquid	Sun
Argentina	ene-02	*A. brasilense* Az39	Laboratorios Arbo SRL	Liquid	W, C, G
Argentina	Rhizoflo Premium	*A. brasilense* Az39	Laboratorios CKC SA	Liquid	W, C, Sun, S, Soy
Argentina	Rhizoflo Liquid Trigo	*A. brasilense* Az39	Laboratorios CKC SA	Liquid	W
Argentina	Rhizoflo Liquid Maiz	*A. brasilense* Az39	Laboratorios CKC SA	Liquid	C
Argentina	Azo LQ	*A. brasilense* Az39	Lanther Quimica SA	Liquid	W, C
Argentina	Marketing Agricola	*A. brasilense* Az39	Marketing Agricola SRL	Liquid	W, C, Sun
Argentina	Nitragin Maiz	*A. brasilense* Az39	Monsanto Argentina SAIC	Liquid	C
Argentina	Nitragin Wave	*A. brasilense* Az39	Monsanto Argentina SAIC	Liquid	W, Wg
Argentina	Azotrap	*A. brasilense* Az39	Nitrap SRL	Liquid	W
Argentina	Azotrap Plus	*A. brasilense* Az39	Nitrap SRL	Liquid	C, Sun, L
Argentina	Graminasoil	*A. brasilense* Az39	Nitrasoil Argentina SA	Liquid	C
Argentina	Graminosoil L	*A. brasilense* Az39	Nitrasoil Argentina SA	Liquid	W
Argentina	Bio-Enhance	*A. brasilense* Az39	Nitrasoil Argentina SA	Liquid	Soy
Argentina	Nivel Azo	*A. brasilense* Az39	Nivelagro SA	Liquid	W, C
Argentina	Promozion	*A. brasilense* Az39	Nova SA	Liquid	W, C
Argentina	Nitragin Semillero	*A. brasilense* Az39	Novozymes BioAg SA	Solid	W, C, Sun, Soy
Argentina	Palaversich Biopower	*A. brasilense* Az39	Palaversich y Cia. SAC	Liquid	W, C, S
Argentina	Buscador N	–	Raparo, Angel Ruben	Peat	W, C
Argentina	Azogrowth	*A. brasilense* Az39	Red Surcos SA	Liquid	Cot, Sun,C, Soy, S, W
Argentina	Rizospirilum	*A. brasilense* Az39	Rizobacter SA	Liquid	W, C
Argentina	Zaden Gramineas	*A. brasilense* Az39	Semillera Guasch SRL	Liquid	W
Argentina	Nitrofull G	*A. brasilense* Az39	Serv-Quim SA	Liquid	W
Argentina	Noctin Azo	*A. brasilense* Az39	Sintesis Quimica SAIC	Liquid	W, C, Sun
Argentina	Vigor Part B	*A. brasilense* Az39	Sintesis Quimica SAIC	Liquid	–
Argentina	Masterfix L	*A. brasilense* Az39	Stoller Biociencias SRL	Liquid	W, C
Argentina	Azzea Uno	*A. brasilense* Tuc 27/85	Tres E	Liquid	C
Argentina	Phoebus	*A. brasilense* Az39 (comb.)	Agro Advance Technology S.A.	Liquid	W, C, Sun, Sor
Argentina	PGPR	*A. brasilense* Az39 (comb.)	Green Quality S.A.	Liquid	W, C, Sun, Soy
Argentina	Palaversich Biopower	*A. brasilense* Az39 (comb.)	Palaversich y CIA, SAC	Liquid	W, C, Sor
Argentina	Triagron	*A. brasilense* Az39 (comb.)	Green Quality SA	Liquid	W
Argentina	Fostrigon	*A. brasilense* Az39 (comb.)	Green Quality SA	Liquid	W, C, Sor
Argentina	Biofert Masterfil PGPR	*A. brasilense* Az39 (comb.)	MASFERTIL SA	Liquid	Clo
Argentina	Biomix	*A. brasilense* Az39 (comb.)	FACYT SA	Liquid	Soy
Argentina	Az + Psf	*A. brasilense* Az39 (comb.)	FACYT SA	Liquid	W, C, Sun
Argentina	Nitrap Max Cubo	*A. brasilense* Az39 (comb.)	Nitrap SRL	Liquid	Soy
Argentina	Nitragin Doble	*A. brasilense* Az39 (comb.)	Monsanto Argentina SAIC	Liquid	W

Table 7
Biological products formulated with *Azospirillum* sp. registered in Brazil at 2015. Source: ANPII and EMBRAPA (unpublished data). References: C: corn; W: wheat; R: rice.

Origin	Brand	Active principle	Company	Carrier	Crop
Argentina	Rizospirillum	–	Rizobacter	Liquid	C, W
Brazil	Grap Nod A	*A. brasilense* AbV5 + Abv6	Agrocete	Liquid	C, W
Brazil	Gelfix Gramíneas	*A. brasilense* BR11005 (Sp45)	BASF	Liquid	C
Brazil	Biomax Milho	*A. brasilense* AbV5	Bio Soy	Liquid	C
Brazil	Azzofix	*A. brasilense* AbV5 + Abv6	Microquímica	Liquid	C
Brazil	Nitro1000 Gramíneas	*A. brasilense* AbV5 + Abv6	Nitro1000	Liquid	C, W
Brazil	Nodugran L	*A. brasilense* AbV5 + Abv6	NoduSoy	Liquid	C
Brazil	Azomax	*A. brasilense* AbV5 + Abv6	Novozymes BioAg	Liquid	C
Brazil	Masterfix Gramíneas	*A. brasilense* AbV5 + Abv6	Stoller	Liquid	C, W, R
Brazil	Masterfix Gramíneas	*A. brasilense* AbV5 + Abv6	Stoller	Peat	C, W, R
Brazil	Azototal	*A. brasilense* AbV5 + Abv6	Total Biotecnologia	Liquid	C, W
Brazil	Azototal	*A. brasilense* AbV5 + Abv6	Total Biotecnologia	Peat	C, W

Table 8
Biological products formulated with *Azospirillum* sp. registered in Uruguay at 2015. Source: MAGyP (unpublished data). References: C: corn; W: wheat; S: sorghum.

Origin	Brand	Active principle	Company	Carrier	Crop
Argentina	Nitragin Maíz	*A. brasilense* Az39	Novozymes BioAg SA	Liquid	C
Uruguay	Bioprom	*A. brasilense* Az39	Calister SA	Liquid	C, W
Uruguay	Graminosoil	*A. brasilense* Az39 + CFN535	Lage y Cia. SA	Liquid	C, S

Table 9
Biological products formulated with *Azospirillum* sp. registered in Paraguay at 2015. SENAVE (Paraguay), ANPII (Brazil) and SENASA (Argentina) (unpublished data). References: C: corn; W: wheat; R: rice. Combined products with other PGPR are referred as comb.

Origin	Brand	Active principle	Company	Carrier	Crop
Argentina	Azp 2000	*A. brasilense* Az39	FPC SA	Liquid	C, W
Argentina	Graminazo	*A. brasilense* Az39	Fragaria SA	Liquid	C
Argentina	Graminante Maíz	*A. brasilense* AzM3	Lab. Alquimia SA	Solid	C
Argentina	Graminante Trigo	*A. brasilense* AzT5	Lab. Alquimia SA	Solid	W
Argentina	Rhizoflo Liquid Maíz	*A. brasilense* Az39	Laboratorios CKC	Liquid	C
Argentina	RhizoFlo Premiun (Comb.)	*A. brasilense* Az39	Laboratorios CKC	Liquid	C
Argentina	Nitragin Maíz	*A. brasilense* Az39	Monsanto	Liquid	C
Argentina	Nitragin Semillero	*A. brasilense* Az39	Monsanto	Liquid	C
Brazil	Gelfix Gramineas	*A. brasilense* BR11005 (Sp45)	BASF	Liquid	C
Brazil	Biomax Premiun	*A. brasilense* AbV5	Bio Soy	Liquid	C
Brazil	Grammy Crop	*A. brasilense* BR11005 (Sp45)	Forquímica	Liquid	C, W, R
Brazil	Masterfix Gramíneas	*A. brasilense* AbV5 + Abv6	Stoller	Liquid	C
Brazil	Azototal	-	Total biotecnologia	Liquid	C, W
Paraguay	Nutrichem (Comb.)	*A. brasilense* Az40	Chemtec	Liquid	C

nitrogen or other nutrients fertilization, inoculation with other benefit microbes, etc. providing better vegetative growth conditions. In general, the contribution measured in terms of grain yield production is greater in summer cereals than in other crops like legumes or winter cereals. Because the benefits on plant (and crop) growth occur mostly during the early growth stages and, in particular, enhancing root growth part of the variable results on grain production are explained for changes in the late season growth conditions.

Part of the current challenges of this promising use of azospirilla inoculants are related to the development of friendly formulations for seed treatments to be used under diverse application, storage handling and environmental conditions. For example, several inoculants are recommended in combination with synthetic seed treatments showing long-term survival of the microbes allowing its use in industrial processes. However, their formulations also show several manufacture limitations because of factors like the need of applying large volume of liquids or the handling and attachment of dry powders. In the other hand, the development of alternative application systems like the delivery of the azospirilla in the seeding furrow simultaneously with the planting operation is seen as a solution to surpass the limitations for on-seed inoculation.

It is well known that the use of *Azospirillum* sp. supports the natural growth of diverse plant species mitigating multiple abiotic stresses and providing a direct contribution not only increasing crop yields but also enhancing the efficacy in the use of diverse production resources (i.e. fertilizers, land, etc.) with extended benefits to the environment. But, it is also needed to promote a strong and coordinated communication program about the already measured benefits of the inoculation with *Azospirillum* sp. strains complementing current extensive (i.e. cereals and legumes), and also intensive (i.e. nursery and transplanting for forestry, vegetables, etc.) crop practices. These communication network should include not only direct users of these products (i.e. growers, consultants and extension agents, public and private researchers and developers, inoculant producers, etc.) but also other actors from rural and urban environments and local regulatory agencies. *Azospirillum* sp. research needs also to intensify their studies about the complementary mechanisms with other beneficial microorganisms as well as the genomic identification of markers that will facilitate strain selection and improvement.

Acknowledgments

The authors acknowledge the collaboration of the following researchers for providing valuable information about registered products (brands, production companies, estimated use, etc.) used in this chapter: Carla Louge (SENASA) and Alejandro Perticari (INTA-IMYZA) from Argentina; María Mayans (MAGyP) from Uruguay and Mariángela Hungria (EMBRAPA) and Solon Cordeiro de Araujo, (ANPII) from Brazil. The authors declare that, although one of the authors is from the Technical Development team of Monsanto BioAg, there is neither censorship of results nor conflict of interest regarding the results presented and the conducted research.

References

Balandreau, J., 2002. The spermosphere model to select for plant growth promoting bacteria. In: Kennedy, I.R., Choudhury, A.T.M.A. (Eds.), Biofertilisers in Action, Rural Industries Research and Development Corporation, Barton (ACT, Australia), pp. 55–63.

Baldani, V., Alvarez, M., Baldani, J., Döbereiner, J., 1986. Establishment of inoculated *Azospirillum* spp. in the rhizosphere and in roots of field grown wheat and sorghum. Plant and Soil 90, 35–46.

Bally, R., Thomas-Bauzon, D., Heulin, T., Balandreau, J., 1983. Determination of the most frequent N_2 fixing bacteria in the rice rhizosphere. Canadian Journal of Microbiology 29, 881–887.

Bashan, Y., de-Bashan, L., 2010. How the plant growth-promoting bacterium *Azospirillum* promotes plant growth. A critical assessment. Advances in Agronomy 108, 77–136.

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

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., Ream, Y., Levanony, H., Sade, A., 1989. Nonspecific responses in plant growth, yield, and root colonization of non cereal crop plants to inoculation with *Azospirillum brasilense* Cd. Canadian Journal of Botany 67, 1317–1324.

Bergersen, F., 1971. Biochemistry of symbiotic nitrogen fixation in legumes. Annual Review of Plant Physiology 22, 121–140.

Bottini, R., Fulchieri, M., Pearce, D., Pharis, R., 1989. Identification of gibberellins A_1, A_3, and Iso-A_3 in cultures of *A. lipoferum*. Plant Physiology 90, 45–47.

Burdman, S., Volpin, H., Kigel, J., Kapulnik, Y., Okon, Y., 1996. Promotion of nod gene inducers and nodulation in common bean (*Phaseolus vulgaris*) roots inoculated with *Azospirillum brasilense* Cd. Applied and Environmental Microbiology 62, 3030–3033.

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, 12–19.

Cassán, F., Perrig, D., Sgroy, V., Masciarelli, O., Penna, C., Luna, V., 2009a. *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E 109 promote seed germination and early seedling growth, independently or co-inoculated in maize (*Zea mays* L.) and soybean (*Glycine max* L.). European Journal of Soil Biology 45, 28–35.

Cassán, F., Spaepen, S., Vanderleyden, J., 2010. Indole-3-acetic acid biosynthesis by *Azospirillum brasilense* Az39 and its regulation under biotic and abiotic stress conditions. Abstract of 20th International Conference on Plant Growth Substances, pp. 85.

Cassán, F., Vanderleyden, J., Spaepen, S., 2014. Physiological and agronomical aspects of phytohormone production by model plant growth promoting rhizobacteria (PGPR) belonging to the genus *Azospirillum*. Journal of Plant Growth Regulation 33, 440–459.

Charyulu, P., Fourcassie, F., Barbouche, A., Rondro Harisoa, L., Omar, A., Weinhard, P., Marie, R., Balandreau, J., 1985. Field inoculation of rice using *in vitro* selected bacterial and plant genotypes. In: Klingmüller, W. (Ed.), Azospirillum III: Genetics, Physiology, Ecology. Springer-Verlag, Berlin, Germany, pp. 163–179.

Cohen, A., Bottini, R., Piccoli, P., 2008. *Azospirillum brasilense* Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in arabidopsis plants. Plant Growth Regulation 54, 97–103.

Creus, C., Graziano, M., Casanovas, E., Pereyra, A., 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, C., Sueldo, R., Barassi, C., 1997. Shoot growth and water status in *Azospirillum*-inoculated wheat seedlings grown under osmotic and salt stresses. Plant Physiology and Biochemistry 35, 939–944.

Dardanelli, M., Fernandez de Cordoba, F., Espuny, M., Rodriguez Carvajal, M., Soria Diaz, M., Gil Serrano, A., Okon, Y., Megias, M., 2008. Effect of *Azospirillum brasilense* coinoculated with *Rhizobium* on *Phaseolus vulgaris* flavonoids and Nod factor production under salt stress. Soil Biology and Biochemistry 40, 2713–2721.

de Morais, T., de Brito, C., Brandao, A., Resende, W., 2016. Inoculation of maize with *Azospirillum brasilense* in the seed furrow. Revista Ciência Agronômica 47, 290–298.

Díaz-Zorita, M., Baliña, R.M., Fernández-Canigia, M.V., 2012a. *Azospirillum brasilense* enhances alfalfa productivity: field evaluation. In: Pérez, J.C., Soler Arango, J., Posada Uribe, L.F. (Eds.), PGPR. 9th International and 1st Latinamerican PGPR Workshop. “Returning to Our Roots”. Quirama, Medellín (Colombia), June 3rd–8th 2012.

Díaz-Zorita, M., 2012. Avaliação da produção de milho (*Zea mays* L.) inoculado com *Azospirillum brasilense* na Argentina (Evaluation of corn production (*Zea mays* L.) inoculated with *Azospirillum brasilense* in Argentina). In: Zagatto Paterniani, M.E.A.G., Pereira Duarte, A., Tsunechiro, A. (Eds.), Diversidade e Inovações na Cadeia Produtiva de Milho e Sorgo na Era dos Transgênicos (Diversity and Innovation in the production chain of corn and sorghum in the age of transgenics). Instituto Agronômico, Associação Brasileira de Milho e Sorgo, Campinas (SP, Brazil), pp. 529–536.

Díaz-Zorita, M., Fernández-Canigia, M.V., 2009. Field performance of a liquid formulation of *Azospirillum brasilense* on dryland wheat productivity. European Journal of Soil Biology 45, 3–11.

Díaz-Zorita, M., Micucci, F.G., Fernández-Canigia, M.V., 2012b. Field performance of a seed treatment with *Azospirillum brasilense* on corn productivity. In: Pérez, J.C., Soler Arango, J., Posada Uribe, L.F. (Eds.), PGPR. 9th International and 1st Latinamerican PGPR Workshop. “Returning to Our Roots”. Quirama, Medellín (Colombia), June 3rd–8th 2012.

Díaz-Zorita, M., Fernández-Canigia, M.V., Bravo, O.A., Berger, A., Satorre, E.H., 2015. Field evaluation of extensive crops inoculated with *Azospirillum* sp. In: Cassán, F.D., Okon, Y., Creus, C.M. (Eds.), Handbook for *Azospirillum*, Technical Issues and Protocols. Springer Int. Publishing, Switzerland, pp. 435–445.

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 on wheat. Plant Soil 212, 155–164.

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

Elbeltagy, A., Nishioka, K., Sato, T., Suzuki, H., Ye, B., Hamada, T., Isawa, T., Mitsui, H., Minamisawa, K., 2001. Endophytic colonzation and *in planta* nitrogen fixation by a *Herbaspirillum* sp. Isolated from wild rice species. Applied and Environmental Microbiology 67, 5285–5293.

El Habbasha, S., Tawfik, M., El Kramany, M., 2013. Comparative efficacy of different bio-chemical foliar applications on growth, yield and yield attributes of some wheat cultivars. World Journal of Agricultural Sciences 9, 345–353.

Esquivel-Cote, R., Ramírez-Gama, R., Tsuzuki-Reyes, G., Orozco-Segovia, A., Huante, P., 2010. *Azospirillum lipoferum* strain AZm5 containing 1-aminocyclopropane-1-carboxylic acid deaminase improves early growth of tomato seedlings under nitrogen deficiency. Plant Soil 33, 65–75.

Fukami, J., Nogueira, M.A., Araujo, R.S., Hungria, M., 2016. Accessing inoculation methods of maize and wheat with *Azospirillum brasilense*. AMB Express 6 (3), 13. http://dx.doi.org/10.1186/s13568-015-0171-y.

García de Salamone, I.E., Dobereiner, J., 1996. Maize genotype effects on the response to *Azospirillum* inoculation. Biology and Fertility of Soils 21, 193–196.

García de Salomone, I.E., 2012. Microorganismos promotores del crecimiento vegetal (Microorganisms plant growth promoters). IAH 5, 12–16.

Gassman, W., Appel, H.M., 2016. The interface between abiotic and biotic stress responses. Journal of Experimental Botany 67, 2023–2024.

Giller, K.E., Merckx, R., 2003. Exploring the boundaries of N_2-fixation in non-legumes: an hypothetical and experimental framework. Symbiosis 35, 3–17.

Govindarajan, M., Balandreau, J., Kwon, S., Weon, H., Lakshminarasimhan, C., 2008. Effects of the inoculation of *Burkholderia vietnamensis* and related endophytic diazotrophic bacteria on grain yield of rice. Microbial Ecology 55, 21–37.

Horemans, S., Koninck, K., Neuray, J., Hermans, R., Vlassak, K., 1986. Production of plant growth substances by *Azospirillum* sp. and other rhizophere bacteria. Symbiosis 2, 341–346.

Hungria, M., 2011. Inoculação com *Azospirillum brasilense*: inovação em rendimento a baixo custo (Inoculation with *Azospirillum brasilense*: yield innovation at low cost). EMBRAPA Soja, Londrina (PR, Brazil), p. 38. Documentos 325.

Hungria, M., Nogueira, M.A., Silva Araujo, R., 2013. Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. Biology and Fertility of Soils 49, 791–801.

Islam, M., Sattar, M., Ashrafuzzaman, M., Saud, H., Uddin, M., 2012. Improvement of yield potential of rice through combined application of biofertilizer and chemical nitrogen. African Journal of Microbiology Research 6, 745–750.

Kaneko, T., Minamisawa, K., Isawa, T., Nakatsukasa, H., Mitsui, H., 2010. Complete genomic structure of the cultivated rice endophyte *Azospirillum* sp. B510. DNA Research 17, 37–50.

Kapulnik, Y., Kigel, J., Okon, Y., Nur, I., Henis, Y., 1981. Effect of *Azospirillum* inoculation on some growth parameters and N content of wheat, sorghum and panicum. Plant Soil 61, 65–70.

Kapulnik, Y., Okon, Y., Henis, Y., 1985. Changes in root morphology of wheat caused by *Azospirillum* inoculation. Canadian Journal of Microbiology 31, 881–887.

Kennedy, I., Pereg-Gerk, L., Wood, C., Deaker, R., Gilchrist, K., Katupitiya, S., 1997. Biological nitrogen fixation in nonleguminous field crops: facilitating the evolution between *Azospirillum* and wheat. Plant Soil 194, 65–79.

Khalid, A., Sultana, S., Arshad, M., Mahmood, S., Mahmood, T., Siddique, M., 2011. Performance of auxin producing rhizobacteria for improving growth and yield of wheat and rice grown in rotation under field conditions. International Journal of Agriculture & Applied Sciences 3, 44–50.

Kolb, W., Martin, P., 1985. Response of plant roots to inoculation with *Azospirillum brasilense* and to application of indoleacetic acid. In: Klingmüller, W. (Ed.), *Azospirillum* III: Genetics, Physiology, Ecology. Springer, Berlin (Germany), pp. 215–221.

Lana, M., Dartora, J., Marini, D., Hann, J., 2012. Inoculation with *Azospirillum*, associated with nitrogen fertilization in maize. Revista Ceres Viçosa 59, 399–405.

Mathesius, U., Shalaman, H., Meijer, D., Lugtenberg, B., Spaink, H., Weinman, J., Rodam, L., Sautter, C., Rolfe, B., Djordjevic, M., 1997. New tools for investigating nodule initiation and ontogeny: spot inoculation and microtargeting of transgenic withe clover roots shows auxin involvement and suggest a role for flavonoids. In: Stacey, G., Mullin, B., Gresshoff, P. (Eds.), Advances in Molecular Genetics of Plant–microbe Interactions. Kluwer Academic, Dordrecht (Netherlands).

Martínez-Morales, L., Soto-Urzua, L., Baca, B., Sanchez-Ahedo, J., 2003. Indole-3-butyric acid (IBA) production in culture medium by wild strain *Azospirillum brasilense*. FEMS Microbiology Letters 228, 167–173.

Mehnaz, S., Kowalik, T., Reynolds, B., Lazarovits, G., 2010. Growth promoting effects of corn (*Zea mays*) bacterial isolates under greenhouse and field conditions. Soil Biology and Biochemistry 42, 1848–1856.

Naiman, A., Latrónico, A., García de Salamone, I., 2009. Inoculation of wheat with *Azospirillum brasilense* and *Pseudomonas fluorescens*: impact on the production and rhizosphere microflora. European Journal of Soil Biology 45, 44–51.

Naseri, R., Moghadam, A., Darabi, F., Hatami, A., Tahmasebei, G., 2013. The effect of deficit irrigation and *Azotobacter chroococcum* and *Azospirillum brasilense* on grain yield, yield components of maize (S.C. 704) as a second cropping in western Iran. Bulletin of Environment, Pharmacology and Life Sciences 2, 104–112.

Nelson, L., Knowles, R., 1978. Effect of oxygen and nitrate on nitrogen fixation and denitrification by *Azospirillum brasilense* growth in continuous culture. Canadian Journal of Microbiology 24, 1395–1403.

Okon, Y., 1982. *Azospirillum*: physiological properties, mode of association with roots and its application for the benefit of cereal and forage grass crops. Israel Journal of Botany 31, 214–220.

Okon, Y., Heytler, P., Hardy, W., 1983. N_2 Fixation by *Azospirillum brasilense* and its incorporation into host *Setaria italica*. Applied and Environmental Microbiology 46, 694–697.

Okon, Y., Labandera-Gonzalez, C., 1994. Agronomic applications of *Azospirillum*: an evaluation of 20 years' worldwide field inoculation. Soil Biology and Biochemistry 26, 1591–1606.

Okumura, R.S., de Cinque, M.D., Dallacort, R., Nogueira de Albuquerque, A., da Silva Lobato, A.K., Silva Guedes, E.M., Ferreira de Oliveira Neto, C., Oliveira da Conceição, H.E., Ruffeil Alves, G.A., 2013. *Azospirillum*: a new and efficient alternative to biological nitrogen fixation in grasses. Journal of Food, Agriculture and Environment 11, 1142–1146.

Ona, O., Smets, I., Gysegom, P., Bernaerts, K., Impe, J.V., Prinsen, E., Vanderleyden, J., 2003. The effect of pH on indole-3-acetic acid (IAA) biosynthesis of *Azospirillum brasilense* sp7. Symbiosis 35, 199–208.

Ozturk, A., Caglar, O., Sahin, F., 2003. Yield response of wheat and barley to inoculation of plant growth promoting rhizobacteria at various levels of nitrogen fertilization. Journal of Plant Nutrition and Soil Science 166, 262–266.

Pedrosa, F., Yates, G., 1984. Regulation of nitrogen fixation (*nif*) genes of *Azospirillum brasilense* by *nifA* and *ntrC (glnG)* type genes. FEMS Microbiology Letters 23, 95–101.

Perrig, D., Boiero, L., Masciarelli, O., Penna, C., Cassán, F., Luna, V., 2007. Plant growth promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and their implications for inoculant formulation. Applied Microbiology and Biotechnology 75, 1143–1150.

Piccoli, P., Bottini, R., 1996. Light enhancement of gibberellin production by *Azospirillum lipoferum* cultures. Biocell 20, 200–207.

Prinsen, E., Costacurta, A., Michiels, K., Vanderleyden, J., Van Onckelen, H., 1993. *Azospirillum brasilense* indole-3-acetic acid biosynthesis: evidence for a non-tryptophan dependent pathway. Molecular Plant-Microbe Interactions Journal 6, 609–615.

Puente, M., Li, C., Bashan, Y., 2004. Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II. Growth promotion of cactus seedlings. Plant Biology 6, 643–650.

Remans, R., Beebe, S., Blair, M., Marique, G., Tovar, E., Rao, I., Croonenberghs, A., Torres-Gutierrez, R., El-Idoweity, M., Michiels, J., Vanderlyden, J., 2008a. Physiological and genetic analysis of root responsiveness to auxin-producing plant growth promoting bacteria in common bean (*Phaseolus vulgaris* L.). Plant Soil 302, 149–161.

Remans, R., Schelkens, S., Hernandez, G., Garcia, A., Luis Reyes, J., Mendez, N., Toscano, V., Mulling, M., Galvez, L., Vanderleyden, J., 2008b. Effect of *Rihzobium-Azospirillum* coinoculation on nitrogen fixation and yield of two contrasting *Phaseolus vulgaris* L. genotypes cultivated across different environments in cube. Plant Soil 312, 25–37.

Rivera Botia, D., Revale, S., Molina, R., Gualpa, J., Puente, M., Maroniche, G., Paris, G., Baker, D., Clavijo, B., McLay, K., Spaepen, S., Perticari, A., Vazquez, M., Wisniewski-Dyé, F., Watkins, C., Martínez-Abarca Pastor, F., Vanderleyden, J., Cassán, F., 2014. Complete genome sequence of the model rhizosphere strain *Azospirillum brasilense* Az39, successfully applied in agriculture. Genome Announcements 2 (4). http://dx.doi.org/10.1128/genomeA.00683-14 e00683–14.

Rodriguez Cáceres, E.A., Di Ciocco, C.A., Carletti, S.M., 2008. 25 años de investigación de *Azospirillum brasilense* Az39 en Argentina (25 years of research about *Azospirillum brasilense* Az39 in Argentina). In: Cassán, F.D., García de Salamone, I. (Eds.), *Azospirillum* sp.: Cell Physiology, Plant Interactions and Agronomic Research in Argentina. Asociación Argentina de Microbiología, Buenos Aires (Argentina), pp. 179–188.

Rubio, G., Díaz-Zorita, M., 2015. La tarea de detectar efectos de compuestos biológicos sobre el rendimiento de cultivos (The task of detecting the effects of biological compounds on crop yields). IAH 20, 24–28.

Sant'Anna, F., Almeida, L., Cecagno, R., Reolon, L., Siqueira, F., Machado, M., Vasconcelos, A., Schrank, I., 2011. Genomic insights into the versatility of the plant growth-promoting bacterium *Azospirillum amazonense*. BMC Genom. 12, 409.

Saubidet, M.I., Fatta, N., Barneix, A.J., 2002. The effect of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization by wheat plants. Plant and Soil 245, 215–222.

Schmidt, W., Martin, P., Omay, H., Bangerth, F., 1988. Influence of *Azospirillum brasilense* on nodulation of legumes. In: Klingmüller, W. (Ed.), *Azospirillim* IV. Genetics, Physiology, Ecology. Springer, Heidelberg (Germany), pp. 92–100.

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.

Star, L., Matan, O., Dardanelli, M., Kapulnik, Y., Burdman, S., Okon, Y., 2012. The *Vicia sativa* spp. nigra-*Rhizobium leguminosarum* bv. viciae symbiotic interaction is improved by *Azospirillum brasilense*. Plant Soil 356, 165–174.

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

Strzelczyk, E., Kamper, M., Li, C., 1994. Cytocinin-like-substances and ethylene production by *Azospirillum* in media with different carbon sources. Microbiol Research 149, 55–60.

Tarrand, J., Krieg, N., Dobereiner, J., 1978. A taxonomic study of the *Spirillum lipoferum* group, with descriptions of a new genus, *Azospirillum* gen. nov., and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasilense* sp. nov. Canadian Journal of Microbiology 24, 967–980.

Thiman, K., 1936. On the physiology of the formation of nodule in legumes roots. Proceedings of the National Academy of Sciences USA 22, 511–514.

Thuler, D., Floh, E., Handro, W., Barbosa, H., 2003. Plant growth regulators and amino acids released by *Azospirillum* sp. In chemically defined media. Letters in Applied Microbiology 37, 174–178.

Tien, T., Gaskins, M., Hubbell, D., 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.

Veresoglou, S., Menexes, G., 2010. Impact of inoculation with *Azospirillum* spp. on growth properties and seed yield of wheat: a meta-analysis of studies in the ISI Web of Science from 1981 to 2008. Plant Soil 337, 469–480.

Wisniewski-Dyé, F., Borziak, K., Khalsa-Moyers, G., Alexandre, G., Sukharnikov, L., 2011. *Azospirillum* genomes reveal transition of bacteria from aquatic to terrestrial environments. PLoS Genetics 7 (2), e1002430.