academicJournals

Vol. 17(18), pp. 574-585, 2 May, 2018
DOI: 10.5897/AJB2017.16333
Article Number: E28B95056898
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Review

Azospirillum spp. potential for maize growth and yield

Lucas Tadeu Mazza Revolti*, Carlos Henrique Caprio, Fábio Luíz Checchio Mingotte and Gustavo Vitti Môro

Department of Crop Science, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (UNESP), Jaboticabal-SP-Brazil.

Received 18 November, 2017; Accepted 18 April, 2018

The importance of biotechnology involved in the availability of nutrients to plants in different production systems is well known. In the search for agricultural sustainability, biological nitrogen fixation process stands out, especially in tropical regions where soil organic matter can be rapidly mineralized. In this aspect, researches have pointed out the potentialities of the use of diazotrophic bacteria, as well as other growth-promoting bacteria in *Poaceae*. Maize crop, especially, stands out in the international scenario, requiring a deepening of the research aiming to raise the contribution potential of microorganisms including *Azospirillum* spp. in reducing the consumption of fertilizers from non-renewable sources while promoting an increase in agricultural productivity and mitigating environmental impacts.

Key words: *Zea mays*, diazotrophic bacteria, nitrogen, biological fixation, growth promoting.

INTRODUCTION

Maize (*Zea mays* L.), one of the most important cereals grown throughout the world, is used as a source of food for humans, animal feed and as raw material for industries. The United States of America is the world's largest producer of this crop, followed by China and Brazil, respectively (USDA, 2018). The maize crop is generally influenced by environmental stress problems, among which are those related to low soil fertility, mainly in terms of nitrogen (N) availability (Novakowiski et al., 2011). In this way, nitrogen fertilizers are commonly used in maize crops in order to supply the deficiency of this nutrient in the soil, meet the physiological needs of the plant and provide high yields. However, due to the economic and environmental cost of industrial manufacturing processes for the increasing food demand, it is necessary to develop technological innovations and incorporate them into the agricultural activity aiming to rationalize the use of nitrogen fertilizers. Among the viable alternatives, the use of beneficials generated by the prokaryotic microorganisms is able to fix atmospheric nitrogen and make them available to the plants through association with the plant roots. The microorganisms with these features are representatives of several bacterial phylogenetic groups, called diazotrophic, capable of colonizing both the interior of the roots and the rhizosphere of plants. This type of symbiosis generates a number of benefits including the stimulus to root growth, making it more voluminous and so absorb larger

*Corresponding author. E-mail: lucasmrevolti@gmail.com.

quantities of water and nutrients (Martínez-Morales et al., 2003). Probably because of the higher root volume and better plant nutrition, there are also several reports of higher tolerance to plant pathogens (Correa et al., 2008).

Usually, inoculation with *Azospirillum brasilense* provides an increase of dry mass, N accumulation in plants and grain yield, especially if there is correlation between bacteria and unimproved genotypes under conditions of low N availability (Okon and Vanderleyden, 1997). In addition, plant nutritional status, exudate quality, competing microorganisms and strain choice are also factors that may influence the interaction between the maize plant and the bacterium as well as the efficiency of biological nitrogen fixation (BNF) (Quadros, 2009). Due to the incompatibility of *A. brasilense* with the chemical products used in seed treatment (Croes et al., 1993), there is a need to study alternative methods of seed inoculation. Thus, a method that has been developed is the inoculation of bacteria in the sowing furrow. According to Basi et al. (2011), inoculation with *A. brasilense* (Abv5/Abv6 strains) in the seeds or on the sowing furrow increased the yield of the maize crop independently of the N rate applied in topdressing (Pereira et al., 2015).

In addition to the increase in maize productivity, there are reports in the literature about nitrogen fertilizer economics when the crop is submitted to inoculation with *A. brasilense* (Cheng et al., 2011). The researches related to the efficiency of the use of inoculants based on *A. brasilense* were neglected for many years due to the inconsistency of the results, however, recently they have been more focused on the necessity for development of more sustainable agriculture. Gramineae have some advantages when compared to Leguminosae. They have a fasciculated root system, having advantages over the pivotal system of legumes to extract water and nutrients from the soil, which, together with other physiological factors, promotes greater photosynthetic activity. Therefore, the interest in the biological fixation in Gramineae is great. Not all of the nitrogen required in maize is provided by the bacteria, making the technique a form of N supplementation for the crop. However, this alternative may lead to a reduction in the use of nitrogenous fertilizers and this cost may be equal to or higher than legumes that may be self-sufficient for nitrogen (Döbereiner, 1992). In this way, several investigations involving the association of maize genotypes with *A. brasilense* found positive results, so that Hungria (2011) obtained yield increases of 30% in relation to the uninoculated control and without nitrogen topdressing fertilization when maize genotypes were inoculated with *A. brasilense* whereas Braccini et al. (2012) observed a 20% increase in grain yield under the same conditions. Müller et al. (2016) obtained an increase of up to 28% of productivity in maize, when plants were inoculated with *A. brasilense* at different doses of N. Portugal et al. (2016) verified higher yields in the four N doses tested (0, 30, 60 and 90 kg ha^{-1}) by spraying *A. brasilense* on maize leaves.

However, the adoption of this technology in the agricultural systems is still incipient, because the results vary according to the cultivar, edaphoclimatic conditions, inoculation types and methodology of conduction of the research. Genotypes may interfere with nitrogen uptake, so the identification, selection, and use of more efficient maize genotypes in nitrogen uptake and assimilation is an important strategy, as they contribute to the development of the crop, as well as reduce the contamination of the environment by nitrogen residues (Reis Júnior et al., 2000). Thus, to find responsive maize genotypes to the inoculation with bacteria of the genus *Azospirillum*, associating them with genetic and breeding programs aiming to increase grain production are really important for the development of new cultivars.

MAIZE CROP

Maize (*Zea mays* L.) which belongs to the family of Gramineae (Poaceae), is a monoic, allogamous, annual, robust, erect and diploid (2n = 2x = 20) crop (Paterniani and Campos, 1999). It originates from the teosinte, a Mexican subspecies (*Zea mays* ssp. Mexicana (Schrader) Iltis), which has been cultivated in many parts of the world for more than 8,000 years (the United States of America, China, India, Brazil, France, Indonesia, South Africa and others). It is a polytypic species, being probably one of the greater genetic variability between the cultivated plants. There is genetic variability for practically all traits of the plant and the maintenance of this variability is due to the establishment of germplasm banks, which maintains the diversity of individualized types and under controlled conditions of races (Fornasieri Filho, 2007).

Botanically, maize belongs to the group of C4 plants, its root system is the fasciculate type reaching up to 3 m deep in the soil, while a great part of the roots are in the 0 to 30 cm layer. The stem is a full culm type, consisting of nodes and internodes, while its lanceolate leaves are inserted alternately in the stem, in addition to presenting male inflorescences, tassel, and female inflorescences and ear, and the fruit being classified as cariopse (Ritchie et al., 1993). The domestication of the maize crop occurred through visual selection in the field, considering important traits such as productivity, resistance to diseases and adaptability, among others, giving rise to varieties known nowadays. Thus, from a Gramineae with several stems, small spikelets and with few seeds, it became an erect plant, with a single culm, monoic, with larger ears containing higher seed quantity and quality.

Maize has a great adaptability, represented by varied genotypes, which allows the cultivation from Ecuador to the limit of temperate lands and from sea level to altitudes above 3,600 m, in tropical, subtropical and temperate climates (Barros and Calado, 2014). It is possible that the maize crop may be the one with the greatest genetic variability among the cultivated plants, since there are genetic differences for practically all the traits of plants, being the maintenance of this variability, kept in germplasm banks, which maintains the diversity of individualized types and under controlled conditions of races (Fornasieri Filho, 2007).

Commercially, there are varieties and maize hybrids on the market. Hybrids are suitable for production of systems that use high technology, being single hybrid, single modified hybrid, triple hybrid, triple modified hybrid and double hybrid. While the varieties are recommended for less technical plantings, being obtained by natural pollination through the selection of groups of plants with desirable characteristics, presenting some variability, but with common genetic characteristics (Cruz et al., 2010).

In socioeconomic terms, maize has an undisputed role in the world, due to its exceptional position among exploited agricultural species (Môro and Fritsche, 2015). According to projections, by 2022, maize grain for animal feed will be the most traded in international markets, accounting for 80% of world trade. This position is consolidated due to maize being the most produced cereal in the world, fundamental for animal and human feed, as well as indispensable and driving of several agroindustrial complexes in function of their productive potential, chemical composition, and nutritional value. Demand for the crop has been particularly high in China, driven mainly by animal growth and industrial processes, which account for over 90% of the country's maize imports (USDA, 2017).

Among the nutrients most required by the maize crop is the N, which is important at the initial stage of development of the plant (second week after emergence), when it is with four leaves fully expanded. It is at this stage that the developing root system already shows a considerable percentage of absorbent hairs and differentiated branches and the addition of N stimulates its proliferation with consequent development of the aerial part. Also at this stage the process of floral differentiation begins, which originates the beginnings of the panicle and ear, as well as, it defines the production potential. This implies the necessity of the availability of at least 30 kg ha^{-1} of N at this stage in order not to limit this physiological event (Fancelli, 1997).

Worldwide, it is estimated that maize will reach 1,038.80 million tons of grain produced in the 2017/18 season. The United States, China, and Brazil are the world's largest producers, respectively, totaling a combined production of 672 million tons (USDA, 2017). Over the last five years, the USA maize production has grown by more than 110 million tons at an average yield of 11,000 kg ha^{-1} (USDA, 2017), surpassing the total Brazilian production, which increased by 25 million tons since 2010 (CONAB, 2017), more than 30% of the total produced by the country (FAO, 2017). From the data presented, it can be inferred that even the crop presenting high productive potential is evidenced by grain yields of up to 16,000 kg ha^{-1} in some countries not only in experimental conditions and also by technified farmers (Cantarella, 1993). Their productivity is complex and depends on the interaction between genetic, environmental factors (Argenta et al., 2001) and well-defined management techniques.

The projections in the coming years are that, there will be a substantial increase in the use of fertilizers in Brazil for attending to the intensification of agriculture and the recovery of degraded areas. Considering Brazilian fertilizer production is insufficient to meet national demand, mostly imported from out of the country, as in the period from January to September 2017, 75% (19,182 tons) of fertilizers usage in Brazilian agriculture came from abroad, indicating an increase of 10.3% in relation to the same period of 2016. There were important nutritional growths in nitrogenous fertilizers of 9.2%, phosphates of 23.6% and potassium of 6.6% (ANDA, 2017).

Biological nitrogen fixation (BNF)

Nitrogen is essential for the proper functioning of plants, as it participates in the composition of the related amino acids, protein, chlorophyll, photosynthesis and many essential enzymes for cell maintenance and development as well as it is present in the processes of ionic absorption, respiration, multiplication and differentiation of cellular and genetic inheritance which are essential for the growth and development of the aerial part and the root system (Marschner, 1995; Epstein and Bloom, 2006; Malavolta, 2006; Grassi Filho, 2010; Taiz and Zeiger, 2013). It is known, for example, that N represents about 40% of the total cost of production of the maize crop (Barros Neto, 2008) and about 50% of this applied nitrogen undergoes the action of ammonia volatilization, denitrification, erosion, microbial immobilization and leaching (Reis Júnior et al., 2010) into the water until it reaches the water table, rivers and lagoons, consequently polluting the environments (Lewis et al., 1984).

BNF involves the transformation of atmospheric nitrogen (N_2) to forms assimilable by the plant: ammonium (NH^{4+}) or nitrate (NO^{3-}) through dinitrogenases enzymes existent in diazotrophic bacteria present in the soil (Novakowiski et al., 2011). This process provides nitrogen compounds directly to plants through associations, or when organisms die and release them into the environment, providing the necessary nitrogen for plant development (Lindemann and Glover, 2003).

According to Rudnik et al. (1997), BNF is a process related to the need of the environment and the fixing species, because of enzyme nitrogenase, which is responsible for the reduction of N_2, is inactive in the presence of ammonia. BNF is a significant process in the agricultural sector, with the biological process contributing most of the fixed N. It is estimated that it provides about 175 million tons of N to the biosphere, or 65% of the total which makes it the second most important biological process on the planet after photosynthesis, along with organic decomposition (Moreira and Siqueira, 2006). However, only biological nitrogen fixation is not able to provide all the necessary N to the development of crops that demand a larger amount of this nutrient, so nutritional supplementation with nitrogen fertilizer formulations is necessary. In this way, it is important to know well the history of the planting area, as well as the predecessor crop, in order to define more accurately the N doses, sources and parceling to be applied (Portugal et al., 2017).

Environments of degraded areas, with poor soils, substrates devoid of organic matter, can be stimulated by BNF, when compared with areas with climax vegetation, provided with rich substrates in organic matter, because the cycling that occurs in these environments guarantees the preservation of metabolism and growth rate (Moreira et al., 2010). BNF is a process that depends on several factors. In order for the bacterium to establish a positive interaction with the plant, it is indispensable to use selected *A. brasilense* strains (Hungria, 2011) capable to compete with the microorganisms already present in the soil. Another factor to be taken into account is the choice of the genotype to be inoculated since the beneficial relationship between the hybrid and the bacterium is determined by the quality of the exudates released by the roots of the plant (Nehl et al., 1996). This phenomenon is known as chemotaxis, where each genotype releases a different amount of exudate with different chemical composition, which may or may not be attractive and serve as a carbon source (malate, pyruvate, succinate, and fructose) for the inoculated bacteria (Quadros, 2009).

As for the survival of this microorganism, it is known that *A. brasilense* has a low ability to survive for prolonged periods of time in most soils. The physicochemical conditions of the soil and the absence of the host plant can directly affect the population of the bacteria (Bashan et al., 1995). However, in unfavorable situations, these bacteria develop protection mechanisms such as cysts formation, poly-β-hydroxybutyrate, and melanin production, favoring their survival (Del Gallo and Fendirik, 1994).

The inoculation with diazotrophic bacteria can alter the root system morphology, a number of radicels and root diameter, probably due to the production of growth promoting substances (auxins, gibberellins, and cytokinins), and not only by BNF (Cavallet et al., 2000). The production of phytohormones helps the growth of plants, and can modify the morphology of the roots, which allows a greater volume of soil exploration and a higher nutrient uptake (Silva et al., 2004), greater tolerance to salinity, dryness (Bashan et al., 2004) and plant pathogens (Correa et al., 2008), resulting in more productive plants (Hungria, 2011). The inoculation with *Azospirillum* is carried out similarly to inoculate soybean seeds with *Bradyrhizobium*. The commercial product can be applied in solid form (as peat) or in liquid form. Also, it is necessary to be cautious of the temperature conditions, not leaving exposed to the sun and without joint application with agrochemicals, since they are living microorganisms (Hungria et al., 2010).

The most common inoculant application method is via seeds. In a study by Portugal et al. (2017) and according to Hungria (2011), seed inoculation associated with the addition of 24 kg ha^{-1} of N at sowing and 30 kg ha^{-1} of N at the flowering stage enable average yields around 7,000 kg ha^{-1}. However, seeding furrow inoculation has been studied as a way of avoiding toxicity of the products used in the treatment of seeds on the bacterium, since some chemicals can disorganize the flagellum used by *A. brasilense* in association with the plant (Croes et al., 1993). According to Basi et al. (2011), the application of *A. brasilense* (Abv5 and Abv6 strains) provided an increase in maize productivity, and the inoculation through the sowing groove did not differ from that in the seeds, showing an efficient application method. However, the selection of strains for inoculant manufacturing still needs a lot of research. There are currently technological packages using plant varieties and efficient bacterial strains, which can supply more than 50% of the N necessary to the plant (Bárbaro et al., 2008).

Plant growth-promoting bacteria: *Azospirillum* spp.

An alternative to achieve high yields of maize, with lower consumption of nitrogen fertilizers, is the inoculation of the crop with bacteria that have the capacity to supply nitrogen to the plants, known as plant growth-promoting bacteria (PGPB) which belong to the phylogenetic groups called diazotrophs (Moreira et al., 2010). PGPB are known as free-living bacteria in the soil, rhizosphere, rhizoplane, and phyllosphere that are beneficial to plants. PGPB endophytes residing within the plant have also been found. They directly affect plant growth by supplying substances that are generally scarce. PGPB may aid to uptake nitrogen nutrition of crops through various mechanisms. They are able to fix atmospheric nitrogen; solubilize phosphorus and iron, and produce plant hormones such as auxins, gibberellins, cytokinins, and ethylene. In addition, they promote higher plant tolerance to stresses, such as drought, high salinity, metal toxicity and pesticide loading (Bashan and De-Bashan, 2010).

Diazotrophs comprise a broad range of prokaryotic microorganisms, including representatives of archebacteria, cyanobacteria, gram-positive and gram-negative bacteria that exhibit great morphological, physiological, genetic and phylogenetic diversity. Such diversity guarantees not only the resilience of the processes that mediate in a given ecosystem but also the occurrence of this, in the most different terrestrial habitats (Moreira and Siqueira, 2006). This kind of bacteria can contribute to plant growth by the following characteristics: nitrogen supply, phytorium production, phosphate solubilization (Pedrinho, 2009), increase the activity of nitrate reductase when they grow endophytically in plants (Cassán et al., 2008), as well as acting as an agent for the biological control of pathogens (Correa et al., 2008). Chavarria and De Melo (2011) report that the use of microorganisms in agricultural practices has become increasing since nitrogen fertilization represents an important element in production costs.

The loss of diversity of soil microorganisms, especially diazotrophs, can alter the population structure of other organisms located along the trophic chain. Vital soil processes such as the decomposition of organic matter and the cycling of nutrients can suffer impacts taking the agricultural system to higher dependence on fertilizers. In this context, the knowledge of the phenotypic diversity and genetic structure of the populations present in the rhizosphere can help in the understanding of how the variations in the environment may be influencing the functionality of these populations.

The diazotrophic bacteria, the most studied PGPB, belonging to the *Azospirillum* genus do not form a symbiosis with the host plant (Bashan and Bashan, 2005), and *Azospirillum* spp. is among the most important bacteria involved in the fixation of N_2 in grasses (Cáceres, 1982). This bacterium is characterized by its rod shape, which are commonly uniflagellated, gram-negative, with characteristic vibratory movement and mixed flagellar pattern (Hall and Krieg, 1984). These microorganisms fit into the group of facultative endophytic diazotrophs, as they colonize both the interior of the roots, where their cells can penetrate into intercellular spaces and lodge, as well as in the external part of the roots, being found in the mucigel present in the rhizosphere of plants and occur frequently in tropical and subtropical soil (Bashan and Levanony, 1990; Baldani et al., 1997).

The *Azospirillum* genus, when inoculated, may not achieve the similar efficiency of the rhizobia-leguminous symbioses in the soil. The contribution of N fixed to Gramineae is around 25 to 50 kg N ha^{-1} $year^{-1}$, equivalent to the average supply of approximately 17% of crop demand (Moreira et al., 2010). Several studies have been carried out to identify microorganisms that have a symbiosis with Gramineae, as occurs in the soybean crop with the bacterium *Bradyrhizobium japonicum*. However, the bacterium *A. brasilense* has a great response potential in association with maize cultivation. The interest in the use of this development-promoting bacterium capable of contributing to plant nutrition has increased and tends to increase in the coming years, due to the high financial value invested annually with fertilizers and in relation to the search for sustainable agriculture (Hungria et al., 2010).

These bacteria have a wide ecological distribution, being found in association with monocotyledonous and dicotyledonous plants (Magalhães and Döbereiner, 1984; Döbereiner and Pedrosa, 1987; Lange and Moreira, 2002). It has been investigated that the effect of *Azospirillum* spp. not only on crop yield but also on the physiological causes the possibly of increase in yield (Bárbaro et al., 2008). It is possible to classify this bacteria as rhizocompetent bacteria, because the survival of this genus in the soil, in the absence of host plants, is related to different physiological mechanisms of protection (Bashan and Levanony, 1990; Del Gallo and Fendrik, 1994; Moreira et al., 2010), they are: melanin production, poly-β-hydroxybutyrate (PHB) and polysaccharides (Del Gallo and Fendrik, 1994), formation of cysts (cell aggregates) and change in cell shape.

The *Azospirillum* genus can act on the vegetative growth through the reduction of nitrate in the roots of the plants (Döbereiner et al., 1995; Cassán et al., 2008). Among the effects of the association between these bacteria and the plants are the biological nitrogen fixation capacity (Fukami et al., 2016), solubilization of inorganic phosphate, production of hormones such as auxins and cytokinins (Tien et al., 1979), gibberellins (Bottini et al., 1989), regulation of ethylene biosynthesis (Strzelczyk et al., 1994), as well as a variety of other bioactive molecules (Perrig et al., 2007); the solubilization of phosphates (Rodriguez et al., 2004); the biological control of pathogens (Correa et al., 2008); and the increase of plant resistance to different abiotic stresses (Yang et al., 2009). One of the most striking effects of inoculation with *A. brasilense* on root morphology is represented by the root hair proliferation, making them more voluminous, and consequently able to absorb larger amounts of water and nutrients (Saikia et al., 2012). The association of diazotrophic bacteria of the *Azospirillum* genus culminates with the increase of maize crop yield (Bashan and De-Bashan, 2010).

Other physiological responses caused by inoculation with *Azospirillum* include the improvement in photo-synthetic parameters of leaves, including chlorophyll content and stomatal conductance, higher proline content in aerial part and roots, improvement in water potential, increase in water content of apoplast, higher cell wall elasticity, higher biomass production and higher plant height (Barassi et al., 2008).

Worldwide, the majority of inoculation evaluating experiments with *Azospirillum* spp. in the maize crop showed increases in grain yield (Kennedy et al., 2004; Kannan and Ponmurugan, 2010). In Brazil, Hungria et al. (2010) when inoculating selected species of *A. brasilense* and *A. lipoferum* in maize and wheat, found increases of 26 and 30% in grain yield of these crops, respectively, as well as increases in P and K uptake by plants. Increases in maize yield were also obtained by Cavallet et al. (2000), Novakowisk et al. (2011), Martins et al. (2012) and Araújo et al. (2014) with the inoculation of

Azospirillum spp in the treatment of seeds, as in the sowing furrow or in foliar application. However, positive responses to increase in productivity are not always obtained with inoculation of the seeds with *Azospirillum* spp. as reported by Campos et al. (1999) in oat and wheat crops and by Müller et al. (2012) with the inoculation of *A.brasilense* in the sowing furrow and the treatment of seeds in the maize crop.

Farinelli et al. (2012) evaluated the agronomic viability of the use of the inoculant (*A. brasilense*) in the treatment of seeds in the maize crop, associated to nitrogen topdressing (0, 90 and 120 kg ha^{-1}). They verified that seed inoculation promoted improvements in the morphological and productive traits of maize and that the highest average of grain yield was achieved with the inoculant powder associated with the application of 120 kg ha^{-1} of N in topdressing. Vazquez et al. (2012) evaluated the effects of *A. brasilense* (liquid, peaty and control without inoculant) and the N rates in topdressing (0, 30, 60 and 120 kg ha^{-1}) on the development of the plant and productivity of maize grains. The researchers found that the use of *A. brasilense* based liquid and peaty inoculant did not interfere with the agronomic traits and grain yield of maize and the fertilization with N applied in topdressing resulted in a linear increase in grain yield and the higher applied dose was not sufficient to obtain the maximum response.

Duarte et al. (2012) evaluated the agronomic performance of two maize hybrids (DKB390YG and 30F35H) as a function of seed inoculation with *Azospirillum* spp. ABV 5 + ABV6 strains and N rates in topdressing (0, 30, 60, 90, 120 and 150 kg ha^{-1}). They concluded that the effects of nitrogen fertilization and inoculation with *Azospirillum* on grain nutrition and grain yield of maize depended on the genetic material, with a positive response of DKB 390YG to the inoculation and a higher response of 30F35H to nitrogen fertilization compared to DKB 390YG. The researchers observed that inoculation increased leaf N concentration, but did not provide partial substitution of nitrogen fertilization in maize crop.

Marini et al. (2015) evaluated the efficiency of inoculation of *A. brasilense* based commercial product via seed treatment (100 ml ha^{-1} of inoculant at a concentration of 2.0×10^8 UFC ml^{-1}), in association with different levels of N topdressing fertilization (0, 40, 80, 120 and 160 kg ha^{-1}) via urea, applied between the V_4 and V_6 stages, in two maize genotypes (30F53 and CD386). They verified that the inoculation provided increases of 11 and 12%, for leaf area and dry matter of maize aerial part, respectively. There was a differentiated response of maize hybrids to most of the analyzed variables. Grain yield data were adjusted to the cubic model, obtaining a higher value in the dose of 160 kg ha^{-1} of N by the 30F53 hybrid, with a linear effect increasing as a function of the N doses applied in the hybrid CD386, with an increase in yield of 14.6 kg ha^{-1} for each kg of N applied to the soil.

Laboratory results indicate that the beneficial effect of *Azospirillum* is probably due to obtaining plants with longer roots and larger seedlings, which present a faster initial growth. In field experiments, more roots (54%), higher dry matter in the aerial part (28%) and higher grain yield (7.1% - average of 221 places) were observed, mainly due to the higher number of grains, since there was no change in mean grain weight (Hungria, 2011). The results of studies with inoculation of this microorganism in maize are contradictory. Apparently, what is verified is that there are different ways to carry out the inoculation, with the main ones directly in the seeds, in the furrow of sowing or in the soil when the plant is already in development. Among the causes of variation of available results, it can be inferred that there may be an effect of the fungicides and insecticides applied to the commercial seeds on *Azospirillum*, the culture phase influences the response, or that there is a differential response of the genotypes used.

Considering the aforementioned, obtaining technical information on the application efficiency of inoculants based on *A. brasilense* via seed treatment, spraying in the interior of seeding furrow or foliar spraying (reaching the neck region), in the V_4 stage, can promote the reduction in the use of nitrogen fertilizers in maize crop, with increases in morphological, agronomic and grain yield components.

In Brazil, it is estimated that the use of inoculants containing selected strains of *A. brasilense* can result in an estimated saving of US$ 2 billion per year, considering fertilizer transport costs (Hungria et al., 2010). Consideration should also be given to the benefits of less environmental pollution resulting from the production and use of nitrogen fertilizers as well as the reduction in the emission of greenhouse gases. Thus, researches involving bacteria of the *Azospirillum* genus is developed by plant breeders, because these microorganisms can associate and provide benefits to crops of great economic importance, such as maize, sorghum, wheat, sugarcane, among others. However, the interaction of maize genotypes with the strains of bacteria of the *Azospirillum* genus is not yet fully elucidated, so research in this sense has been growing steadily in the world.

Genotype × inoculation interaction

Studies related to the interaction between genotypes × inoculation demonstrate that there is a differentiated response of the genotypes when they are inoculated with diazotrophic bacteria. Reis Júnior et al. (2000) pointed out that when BNF is related with non-leguminous species, the effect of plant genotype on N fixation is expressive. Thus, identifying, selecting and using less demanding genotypes for the N element are important tools (Revolti, 2014).

Usually, inoculation with *A. brasilense* provides an increase of dry mass, N accumulation in plants and grain yield, especially if the association is between bacteria and unimproved genotypes and under conditions of low N availability (Okon and Vanderleyden, 1997). In addition to these factors, the nutritional state of the plant, the quality of the exudates, the existence of competing microorganisms and the choice of the adapted strain to each region in terms of climate, management system and cultivars, are also factors that can influence the interaction between maize plant and bacterium and affect the efficiency of BNF (Quadros, 2009). According to Bartchechen et al. (2010), research involving *Azospirillum* in maize indicates that the interaction between the bacterium and the plant varies according to the cultivar, edaphoclimatic conditions, and methodologies of conduction of the trials. These methodologies mainly involve: a) forms of inoculation: seed coating, sowing furrow, application via leaf or plant spray; and b) experimental designs, control of pathogens and pests, and vegetative stage of the plant at the time of inoculation.

However, these bacteria naturally exist in most soils and present wide genetic diversity (Ardakani et al., 2011), making it necessary to use efficient strains in BNF and in the production of growth and development hormones capable to compete with native bacteria as well as to select maize genotypes responsive or suitable for this association (Basi, 2013). Thus, following the Brazilian legislation for inoculants, established by the Brazilian Ministry of Agriculture, Livestock and Supply, Embrapa Soybean researchers led by Hungria (2011) found that Ab-V4, Ab-V5, Ab-V6, and Ab- V7 showed higher soil survival, higher growth promotion and adaptation to technologies used in maize and wheat crops. Following the same study, maize yield increases up to 30% in relation to the control not inoculated with the bacteria. This fact justifies the reason why inoculant manufacturers opt for the Ab-V5 and Ab-V6 strains in their products intended for maize and wheat crops.

In practical terms, there are studies in which 85% of the experiments involving maize and bacteria of the *Azospirillum* genus responded positively, with an average productivity increase of 472 kg ha^{-1} (Díaz-Zorita and Fernandez, 2008). Several experiments conducted in Latin America during the last decades, have indicated in the majority, plant growth and/or productivity of the crops studied (Cassán and García de Salamone, 2008). Costa et al. (2015) studied the inoculation with *A. brasilense* in seeds and nitrogen doses in maize crop in Cerrado region and verified that the use of the bacterium promoted higher plant height, culm diameter, leaf chlorophyll index, culm and root dry mass, ear insertion height, thousand grain weight, and grain yield. Similarly, Cunha et al. (2014) also found positive results when inoculating maize seeds with the bacterium, obtaining an increase in productivity and reduction of nitrogen topdressing application by 16%. Morais et al. (2016), when testing several doses of inoculant containing *A. brasilense* applied to maize sowing, verified that the dose of 200 ml ha^{-1} promoted an increase in grain yield. Verona et al. (2010) observed that the inoculation provided greater culm diameter and greater weight in relation to the aerial part dry mass even in water stress. It is known that in addition to the leaves, most of the reserves produced by the plant are stored in the stalks, making this ratio of greater aerial part mass and larger culm diameter produce better storage conditions and a possible higher final production, since these reserves are indispensable for the good development of the plant, mainly in the reproductive phase, to supply the drains represented by the ears.

The benefits of inoculation of *A. brasilense* can be verified in other cultures. Sala et al. (2008) inoculated wheat seeds (*Triticum aestivum hard* L. and *Triticum durum* L.), providing 0, 60 and 120 kg ha^{-1} of urea (70% at sowing and 30%, 30 days after sowing) and they could see that there is an interaction of the endophytic bacteria response with nitrogen fertilization. As a result of the research, the highest cumulative amount of N was obtained with the inoculation of the *A. brasilense* IAC-AT-8 strain and with the addition of 60 kg ha^{-1} of N when compared to the control. They were also able to prove that when the dose of N increased from 60 to 120 kg ha^{-1}, there was a linear decrease in the nutrient utilization efficiency index.

However, not all of the necessary N in the maize crop provided the bacteria. It is an alternative that allows the producer to reduce the use of nitrogenous fertilizers achieving an economy equal to or greater than that found in legumes, which can be self-sufficient in N (Döbereiner, 1992). García de Salomone and Döbereiner (1996) evaluated different maize genotypes inoculated with *Azospirillum* and obtained different responses regarding the inoculation under the yield in the production, noting that there are variations in the interactions between maize genotypes and diazotrophic bacteria.

Scientific advances regarding the use of *Azospirillum* sp. in maize crop

A summary of the main results obtained from inoculation with *Azospirillum* sp. in recent years is shown in Table 1.

FINAL CONSIDERATIONS

Although there are plans to set up new industries and open up new areas for mineral exploration, the situation over the next ten years is quite critical. Thus, the use of Plant Growth-Promoting Bacteria that assist in the biological fixation of nitrogen is of great value for maize and other grasses, as it is an excellent economic

Table 1. Main recent results obtained from inoculation with *Azospirillum* spp. in the corn crop.

Species / methodology adopted	Inoculation effects	References
Azospirillum lipoferum strain (Accession no. GQ255950)	Mitigation the deleterious effects of drought on maize. Benefits in corn crop in normal as well as drought stress conditions.	Bano et al., 2013.
Seed inoculation with Ab-V5 and Ab-V6 strains of *A. brasilense*	Increase in maize plant height, yield of maize grains and plant's dry matter when compared to control.	Braccini et al., 2012
Liquid inoculant carryng *A. brasilense* Ab-V5	The grain production was increased by 29% in the treatment with *A. brasilense* and nitrogen compared to nitrogen fertilization alone.	Ferreira et al., 2013
Seed inoculation with *A. brasilense* and five rates of N.	Decreasing of Fe concentration in leaves and increase of corn grain yield.	Galindo et al., 2016
Seed inoculation with diferente doses os *A. brasilense* strains Ab-V5 and Ab-V6.	The dose of 200 mL ha^{-1} *Azospirillum* was noteworthy for grain production.	Morais et al., 2016
Seed inoculation with *A. brasilense* Ab-V5 strain coinoculated with *Rhizobium tropici*	Seed inoculation with *A. brasilense* as well as their co-inoculation with *Rhizobium tropici* in the absence of N fertilization was efficient to increase plant growth.	Picazevicz et al., 2017.
Inoculation by pelleting with a mixture of *A. brasilense* Az39 and Az30 and *A. lipoferum* Sp7	The number of seeds per ear was increased ca. 2-fold in the inoculated plants. The dry weight of seeds (kg ha^{-1}) was also increased by 59%.	Fulchieri and Frioni, 1994
Three doses of seed inoculation with *A. brasilense* Ab-V5 and Ab-V6 associated with presence and absence of N fertilization	Half the dose of N fertilizer combined with 150 g per 25kg of seeds of A. brasilense in peat formulation provided significantly superior results in agronomic performance of maize, particularly regarding grain yield, thousand seed weight and dry biomass of both shoot and root.	Garcia et al., 2017
Experiments *in vitro* and bioassays were evaluated studying the capacity of *Azospirillum* sp. and *Pseudomonas* sp. to degrade glyphosate residues both in vitro and in vivo in maize plants (*Zea mays* L.) at different growth stages.	In bioassays, inoculation with both bacteria improved germination and root emergence, primary root growth, root hair development and coleoptile growth in seeds previously treated with the herbicide. Foliar inoculation with *Azospirillum* sp. and *Pseudomonas*sp. in glyphosate-treated plants improved root and shoot biomass and increased foliar area, photosynthetic pigments and phytohormone content as well, thus increasing maize yield in the field while concomitantly decreasing herbicide accumulation in leaves and grains.	Travaglia et al., 2015
Efficiency of *Azospirillum brasilense* MTCC125 flocculated cells with standard grown cells under in vitro conditions and in association with maize (*Zea mays* L.) under field conditions.	Field studies with *A. brasilense* flocculated cells conducted under normal irrigated conditions and by withholding irrigation at 25, 50, and 75% available water-holding capacity (AWHC) showed a significant increase in plant height (19%), plant dry weight (16%), grain yield (31%), stover yield (17%) and nitrogen uptake (18%) compared with standard grown cell treatment.	Joe et al., 2012
Corn seeds were inoculated with a commercial product based on the Ab-V5 and Ab-V6 strains of *A. brasilense*	Inoculation with *A. brasilense* provided increases of 11 and 12% in leaf area and shoot dry matter, respectively.	Marini et al., 2015

Table 1. Contd.

Inoculation with the active strain of *Azospirillum brasilense* (strain 65B) Inoculation was performed just before sowing: bacterium suspension was mixed with maize seeds and used for spraying the field.	Inoculation of maize with *A. brasilense* bacteria contributed to an increase of that plant vigour and yield.	Swędrzyńska and Sawicka, 2000
Seven strains of *A. brasilense* (Ab-V1, Ab-V2, Ab-V4, Ab-V5, Ab-V6, Ab-V7 and Ab-V8) isolated from maize plants and two of *A. lipoferum* (Al-V1 and Al-V2) were aplicated to seeds as peat-based inoculants.	*A. brasilense* strains Ab-V4, Ab-V5, Ab-V6 and Ab-V7 increased grain yields of maize by 662–823 kg ha^{-1}, or 24-30%, in relation to non-inoculated controls.	Hungria et al., 2010
Many strains of *A. brasilense* and *A. lipoferum* have been used to inoculate cultivars of different cultivars of species of plants, in including maize, in more than ten countries.	The data indicates 60-70% occurrence of success with statistically significant increases in yield of the order of 5-30%.	Okon and Labandera-Gonzalez, 1994.
The present study in pots was performed to investigate the effect of inoculation of individual strains (and a mixture) of *Azospirillum* spp., and their nitrate reductase negative (NR-) mutants, on the growth of four of these maize genotypes.	Two maize genotypes produced similar increases in grain yield when they were inoculated with a mixture of *Azospirillum* spp. strains or fertilized with the equivalent of 100 kg N ha^{-1}. The two genotypes showed a large increase in total N accumulation, suggesting that the response was due to increased N acquisition, but not due to bacterial nitrate reductase as the NR- mutants generally caused plant responses similar to those of the parent strains.	De Salamone et al., 1996
Association of doses of nitrogen fertilization with and without inoculation with *A. brasilense* strains Ab-V5 and Ab-V6.	There was a significant increase in both number and mass of commercial corn cobs with A. brasilense inoculation as compared with treatment without inoculation. The association of inoculation with *A. brasilense* and nitrogen increase more than 30% the corn cobs production.	Araújo et al., 2014
Four methods of inoculation with *A. brasilense* (Ab-V5 and Ab-V6) were compared: (1) standard seed inoculation – control treatment; (2) inoculation in the planting furrow at sowing; (3) leaf spray inoculation at the V2.5 stage of the maize plant growth cycle or 3rd tiller for wheat ; and (4) spray inoculation on the soil surface at the V2.5 stage of the maize plant growth cycle or 3rd tiller for wheat.	All inoculation techniques increased the abundance of diazotrophic bacteria in plant tissues, and foliar spray improved colonization of leaves, while soil inoculations favored root and rhizosphere colonization. In field experiments, inoculation with *A. brasilense* allowed for a 25 % reduction in the need for N fertilizers.	Fukami et al., 2016

opportunity to be able to increase the efficiency of nutrient absorption, in addition to providing environmental benefits associated with reduced fertilizer use. However, the efficiency in the use of diazotrophic bacteria in maize is related to the all management involved during the development of the plant, since there is a need to know the physiological characteristics and water needs, nutritional, pest control and crop diseases, so that the actual influence of the growth-promoting bacteria present in the rhizosphere on the plants can be obtained. In addition, the total nitrogen supply of the crop will not only be supplied by the microorganisms, it is necessary to stagger the topdressing fertilization at the recommended doses for the crop, as shown in several types of research.

On this way, considering the existence of a wide range of available genotypes of maize, either commercially or in research institutes, there is a great importance to know the interaction of the genotype under study with the inoculation form of diazotrophic bacteria, in order to identify, select and use less demanding genotypes for the N element. This happens because, as occurs with *A. brasilense* and several microorganisms, there are genetic variations within the same species, which demands the development of research aimed to evaluate and relate more closely maize genotypes under study with the degrees of association of the existing strains. So it will be possible to delineate genetic breeding programs in an efficient way in order to develop cultivars more responsive to the inoculation with diazotrophic bacteria. Thus, in addition to the low cost for farmers, the use of beneficial bacteria containing *Azospirillum* contributes to the environment and may be the subject of future negotiations on carbon credits trading. The prospects are also that, in the coming years, the agronomic efficiency of inoculation with *Azospirillum* can be confirmed with other Gramineae.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

REFERENCES

ANDA - Associação Nacional para Difusão de Adubos (2017). Mercado de Fertilizantes. Jan-Sep 2017. (available at http://www.anda.org.br/). Access in Oct. 20, 2017.

Araújo RM, Araújo ASF, Nunes LAPL, Figueiredo MVB (2014). Resposta do milho verde à inoculação com *Azospirillum brasilense* e níveis de nitrogênio. Cienc. Rural 44(9):1556-1560.

Ardakani MR, Mazaheri D, Mafakheri S, Moghaddam A (2011). Absorption efficiency of N, P, K through triple inoculation of wheat (*Triticum aestivum* L.) by *Azospirillum brasilense*, *Streptomyces* sp., Glomus intraradices and manure application. Physiol. Mol. Biol. Plants 17:181-192.

Argenta G, Silva PRF, Sangoi L (2001). Arranjo de plantas em milho: análise do estado-da-arte. Cienc. Rural 31(6):1075-1084.

Baldani JI, Caruso L, Baldani VLD, Goi SR, Döbereiner J (1997). Recent advances in BFN with non-legume plants. Soil Biol. Biochem. 29:911-922.

Bano Q, Ilyas N, Bano A, Zafar N, Akram A, Hassan F (2013). Effect of Azospirillum inoculation on maize (Zea mays L.) under drought stress. Pak. J. Bot. 45:13-20.

Barassi CA, Sueldo RJ, Creus CM, Carrozzi LE, Casanovas WM, Pereyra MA (2008). Potencialidad de *Azospirillum* en optimizer el crecimiento vegetal bajo condiciones adversas. In: Cassán FD, García de Salamone I. *Azospirillum* spp.: cell physiology, plant interactions and agronomic research in Argentina. Asociación Argentina de Microbiologia, pp. 49-59.

Bárbaro IM, Brancalião SR, Ticelli M (2008). É possível fixação biológica de nitrogênio no milho. INFOBIBOS - Tecnological informations. Online. (available at http://www.infobibos.com/artigos/2008_2/fixacao/index.htm). Access in Oct 22, 2017.

Barros JFC, Calado JG (2014). A cultura do milho. Évora: Univerisade de Évora. 52 p.

Barros Neto CR (2008). Efeito do nitrogênio e da inoculação de sementes com *Azospirillum brasilense* no rendimento de grãos de milho. Ponta Grossa: Universidade Estadual de Ponta Grossa. 29 p.

Bartchechen A, Fiori CCL, Watanabe SH, Guarido RC (2010). Efeito da inoculação de *Azospirillum brasilense* na produtividade da cultura do milho (*Zea mays* L.). Campo Digit. 5(1):56-59.

Bashan Y, Bashan LE (2005). Pant growth-promoting. In. Hillel D (ed) Encyclopedia of soils in the environment, Elsevier, Oxford, UK, 1:103-115.

Bashan Y, De-Bashan LE (2010). How the plant growth-promoting bacterium *Azospirillum* promotes plant growth - a critical assessment. Adv. Agron. 108:77-136.

Bashan Y, Holguin G, De-Bashan LE (2004). *Azospirillum*-plant relations physiological, molecular, agricultural, and environmental advances. Can. J. Microbiol. 50:521-577.

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

Bashan Y, Puente ME, Rodriguez-Medonza MN, Toledo G, Holguin G, Ferrera-Cerrato R, Pedrin S (1995). Survival of *Azospirillum brasilense* in the bulk soil and rhizosphere of 23 soil types. Appl. Environ. Microbiol. 61:1938-1945.

Basi S (2013). Associação de *Azospirillum brasilense* e de nitrogênio em cobertura na cultura do milho. Guarapuava: Universidade Estadual do Centro-Oeste. 50 p.

Basi S, Lopes ECP, Kaminski TH, Pivatto RAD, Cheng NC, Sandini IE (2011). *Azospirillum brasilense* nas sementes e no sulco de semeadura da cultura do milho. In. Semana de Integração, Ensino, Pesquisa e Extensão. Guarapuava: II SIEPE.

Bottini R, Fulchieri M, Pearce D, Pharis R (1989). Identification of gibberellins A1, A3, and iso-A3 in cultures of *A. lipoferum*. Plant Physiol. 90:45-47.

Braccini AL, Dan LGM, Piccinin GG, Albrecht LP, Barbosa MC, Ortiz AHT (2012). Seed inoculation with *Azospirillum brasilense*, associated with the use of bio-regulators in maize. Rev. Caatinga 25(2):58-64.

Cáceres EAR (1982). Improved medium for isolation of *Azospirillim* spp.. Appl. Environ. Microbiol. 44(43):990-991.

Campos BHC, Thiesen S, Gnatta V (1999). Inoculante “Graminante” nas culturas de trigo e aveia. Cienc. Rural 23(3):401-407.

Cantarella H (1993). Calagem e adubação do milho. In. Büll LT, Cantarella H (eds) Cultura do milho: fatores que afetam a produtividade. Piracicaba: Potafos. pp. 147-185.

Cassán F, Sgroy V, Perrig D, Masciarelli O, Luna V (2008). Producción de fitohormonas por *Azospirillum* sp. Aspectos fisiológicos y tecnológicos de la promoción del crecimiento vegetal. In: Cassán FD, García de Salamone I (eds) *Azospirillum* spp.: cell physiology, plant interactions and agronomic research in Argentina. Argentina: Asociación Argentina de Microbiologia. pp. 61-86.

Cassán FD, García de Salamone I (2008). (eds) *Azospirillum* spp.: cell physiology, plant interactions and agronomic research in Argentina. Argentina: Asociación Argentina de Microbiologia. 268 p.

Cavallet LE, Pessoa ACS, Helmich JJ, Helmich PR, Ost CF (2000) Produtividade do milho em resposta à aplicação de nitrogênio e inoculação das sementes com *Azospirillum* spp. Rev. Bras. Eng. Agríc. Ambient. 4:129-132.

Chavarria G, De Mello N (2011). Bactérias do gênero *Azospirillum* e sua relação com gramíneas. Rev. Plantio Direto, 125.

Cheng NC, Novakowiski JH, Sandini I, Domingues L (2011). Substituição da adubação nitrogenada de base pela inoculação com *Azospirillum brasilense* na cultura do milho. In. Seminário Nacional de Milho Safrinha. Lucas do Rio Verde: Fundação Rio Verde. pp. 377-382.

Companhia Nacional de Abastecimento (CONAB) (2017). Acompanhamento da safra brasileira de grãos. 2017/2018 Season. (available at http://www.conab.gov.br/). Access in oct 23, 2017.

Correa OS, Romero AM, Soria MA, De Estrada M (2008). *Azospirillum brasilense*-plant genotype interactions modify tomato response to bacterial diseases, and root and foliar microbial communities. In: Cassán FD, Garcia De Salamone I (eds) Azospirillum spp.: cell physiology, plant interactions and agronomic research in Argentina. Argentina: Associación Argentina de Microbiologia. pp. 87-95.

Costa RRG, Quirino GSF, Naves DCF, Santos CB, Rocha AFS (2015). Efficiency of inoculant with *Azospirillum brasilense* on the growth and yield of second-harvest maize. Pesq. Agropecu. Trop. 45(3):304-311.

Croes CL, Moens S, Van Bastelaere E, Vanderleyden J, Michiels KW (1993). The polar flagellum mediates *Azospirillum brasilense* adsorption to wheat roots. J. Gen Microbiol. 139:2261-2269.

Cruz JC, Pereira Filho IA, Garcia JC, Duarte JO (2010). Cultivo do Milho: Cultivares. Sistema de Produção. 6ed. Sete Lagoas: Embrapa Milho e Sorgo. 8 p.

Cunha FN, Silva NF, Bastos FJC, Carvalho JJ, Moura LMF, Teixeira

MB, Rocha AC, Souchie EL (2014). Efeito da *Azospirillum brasilense* na Produtividade de Milho no Sudoeste Goiano. Rev. Bras. Milho Sorgo. 13:261-272.

De Salamone IG, Dobereiner J, Urquiaga S, Boddey RM (1996). Biological nitrogen fixation in Azospirillum strain-maize genotype associations as evaluated by the 15 N isotope dilution technique. Biol Fertil. Soils 23(3):249-256.

Del Gallo M, Fendrik I (1994). The rhizosphere and *Azospirillum*. In. Okon Y (eds) *Azospirillum* plant association. Boca Raton: CGC Critical Reviews in Plant Science. pp. 57-75.

Díaz-Zorita M, Fernandez Canigia MV (2008). Análisis de la producción de cereales inoculados con *Azospirillum brasilense* en la República Argentina. In. Cassán FD, Garcia de Salamone I (eds) *Azospirillum* spp.: cell physiology, plant interactions and agronomic research in Argentina. Argentina: Asociación Argentina de Microbiologia. pp. 155-166.

Döbereiner J, Baldani VLD, Baldani JI (1995). Como isolar e identificar bactérias diazotróficas de plantas não-leguminosas. Brasília: Embrapa-SPI. 60 p.

Döbereiner J, Pedrosa FO (1987). Nitrogen-fixing bacteria in non-leguminous crop plants. Science Tech, Madison, USA. 155 p.

Döbereiner, J (1992). History and new perspectives of diazotrophs in association with non-leguminous plants. Symbiosis. 13:1-13.

Duarte AP, Piedade RC, Martins VC, Cantarella H, Barros VLNP (2012). Resposta de cultivares de milho ao nitrogênio em cobertura e à inoculação com *Azospirillum*. In. 29 Congresso nacional de milho e sorgo. Campinas: Instituto Agronômico/Associação Brasileira de Milho e Sorgo. pp. 1786-1792.

Epstein E, Bloom AJ (2006). Nutrição mineral de plantas: princípios e perspectivas. Londrina: Editora Planta. 403 p.

Fancelli AL (1997). Cultura do milho: A importância da tecnologia. Informações Agronômicas, Piracicaba. 1(78):4-6.

FAO– Food and Agriculture Organization Statistical Databases (2017). Crops. FAO-Food and Agriculture Organization of the United Nations. (available at http://www.fao.org/home/en/). Access in oct 23, 2017.

Farinelli R, Hanashiro RK, Amaral CB, Fornasieri Filho D (2012). Reposta da cultura do milho à inoculação de sementes e adubação nitrogenada em cobertura. In. 29 Congresso nacional de milho e sorgo. Campinas: Instituto Agronômico/Associação Brasileira de Milho e Sorgo. pp. 1672-1678.

Ferreira AS, Pires RR, Rabelo PG, Oliveira RC, Luz JMQ, Brito CH (2013). Implications of Azospirillum brasilense inoculation and nutrient addition on maize in soils of the Brazilian Cerrado under greenhouse and field conditions. Appl. Soil Ecol. 72:103-108.

Fornasieri Filho D (2007). Manual da cultura do milho. Jaboticabal: Funep. 574 p.

Fukami J, Nogueira MA, Araujo RS, Hungria M (2016). Acessing inoculation methods of maize and wheat with *Azospirillum brasilense*. AMB Express 6:1-13.

Fulchieri M, Frioni L (1994). Azospirillum inoculation on maize (Zea mays): effect on yield in a field experiment in central Argentina. Soil Biol. Biochem. 26(7):921-923.

Galindo FS, Teixeira Filho MCM, Buzetti S, Santini JMK, Alves CJ, Nogueira LM, Ludkiewicz MGZ, Andreotti M, Bellotte JLM (2016). Corn yield and foliar diagnosis affected by nitrogen fertilization and inoculation with Azospirillum brasilense. Rev. Bras. Ciênc. Solo 40:1-18.

García de Salomone, Döbereiner J (1996). Maize genotype effects on the response to *Azospirillum* inoculation. Biol. Fertil. Soils 21(3):193-196.

Garcia MM, Pereira LC, Braccini AL, Angelotti P, Suzukawa AK, Marteli DC, Felber PH, Bianchessi PA, Dametto IB (2017). Effects of Azospirillum brasilense on growth and yield compounds of maize grown at nitrogen limiting conditions. Rev. Ciênc Agrár. 40(2):353-362.

Grassi Filho H (2010). Funções do nitrogênio e enxofre nas plantas. In. Vale DW, Sousa JI, Prado RM (eds) Manejo da fertilidade do solo e nutrição de plantas. Jaboticabal: FCAV, pp. 187-198.

Hall PG, Krieg NR (1984). Application of the indirect immunoperoxidase stain technique to the flagella of *Azospirillum brasilense*. Appl. Environ. Microbiol. 47(2):433-435.

Hungria M (2011). Inoculação com *Azospirillum brasilense*: inovação em rendimento a baixo custo. Londrina: Embrapa.

Hungria M, Campo RJ, Souza EMS, Pedrosa FO (2010). Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. Plant Soil 331(1/2):413-425.

Joe MM, Karthikeyan B, Chauhan PS, Shagol C, Islam MR, Deiveekasundaram M, Sa T (2012). Survival of Azospirillum brasilense flocculated cells in alginate and its inoculation effect on growth and yield of maize under water deficit conditions. Eur. J. Soil Biol. 50:198-206.

Kannan T, Ponmurugan P (2010). Response of paddy (*Oryza sativa* L.) varieties to *Azospirillum brasilense* inoculation. J. Phytol. 2:8-13.

Kennedy IR, Choudhury ATMA, Kecskes ML (2004). Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better explored. Soil Biol. Biochem. 36:1229-1244.

Lange A, Moreira FMA (2002). Detecção de *Azospirillum amazonense* em raízes e rizosfera de Orchidaceae e de outras famílias vegetais. Rev. Bras. Ciênc. Solo 26:535-543.

Lewis WM, Saunders JF, Crumpaker DW, Brendecke CM (1984). Eutrophication and land use, Lake Dillon, Colorado. Ecol. Stud. 46:202.

Lindemann WC, Glover CR (2003). Nitrogen fixation by Legumes. Cooperative extension service. New México State University: College of Agriculture and Homes Economics. Guide A-129.

Magalhães FMM, Döbereiner J (1984). Ocorrência de *Azospirillum amazonense* em alguns ecossistemas da Amazônia. Rev. Microbiol. 15:246-252.

Malavolta E (2006). Manual de nutrição mineral de plantas. Piracicaba: Ceres. 631 p.

Marini D, Guimarães VF, Dartora J, Lana MC, Pinto Júnior AS (2015). Growth and yield of corn hybrids in response to association with *Azospirillum brasilense* and nitrogen fertilization. Rev. Ceres. 62(1):117-123.

Marschner H (1995). Mineral nutrition of higher plants. London: Academic Press. 889 p.

Martínez-Morales LJ, Soto-Urzúa L, Baca BE, Sánchez-Ahédo JA (2003). Indole-3-butyric acid (IBA) production in culture medium by wild strain *Azospirillum brasilense*. FEMS Microbiol. Lett. 228:167-173.

Martins FAD, Andrade AT, Condé ABT, Godinho DB, Caixeta CG, Costa RL, Pomela AWV, Soares CMS (2012). Avaliação de híbridos de milho inoculados com *Azospirillum brasilense*. Pesq. Agropecu. Gau. 18(2):113-128.

Morais TP, Brito CH, Brandão AM, Rezende WS (2016). Inoculation of maize with *Azospirillum brasilense* in the seed furrow. Rev. Cienc. Agron. 47(2):290-298.

Moreira FMS, Da Silva K, Nobrega RSA, Carvalho F (2010). Bactérias diazotróficas associativas: diversidade, ecologia e potencial de aplicações. Comunicata Scientiae 1(2):74-99.

Moreira FMS, Siqueira JO (2006). Microbiologia e bioquímica do solo. 2ed. Lavras: UFLA. 729 p.

Môro GV, Fritsche-Neto R (2015). Importância e usos do milho no Brasil. In. Borém A, Galvão JCC, Pimentel MA (eds) Milho do plantio à colheita. Viçosa: UFV. pp. 9-23.

Müller TM, Bazzanezi AN, Vidal V, Turok JDN, Rodrigues JD, Sandini IE (2012). Inoculação de *Azospirillum brasilense* no tratamento de sementes e sulco de semeadura na cultura do milho. In. 29 Congresso nacional de milho e sorgo. Campinas: Instituto Agronômico/Associação Brasileira de Milho e Sorgo. pp. 1665-1671.

Müller TM, Sandini IE, Rodrigues JD, Novakowiski JH, Basi S, Kaminski TH (2016). Combination of inoculation methods of *Azospirilum brasilense* with broadcasting of nitrogen fertilizer increases corn yield. Cienc. Rural 46(2):210-215.

Nehl DB, Allem SJ, Brown JF (1996). Deleterious rhizosphere bacteria: an integrating perspective. Appl. Soil Ecol. 5:1-20.

Novakowiski JH, Sandini IE, Falbo MK, Moraes A, Novakowiski JH, Cheng NC (2011). Efeito residual da adubação nitrogenada e inoculação de *Azospirillum brasilense* na cultura do milho. Semina: Ciênc. Agrár. 32(1):1687-1698.

Okon Y, Labandera-Gonzales CA (1994). Agronomic applications of Azospirillum: an evaluation of 20 years worldwide field inoculation.

Soil Biol Biochem. 26(12):1591-1601.
Okon Y, Vanderleyden J (1997). Root-associated *Azospirillum* species can stimulate plants. Appl. Environ. Microbiol. 6(7):366-370.
Paterniani E, Campos MS (1999). Melhoramento do milho. In. Borém A (eds) Melhoramento de espécies cultivadas. Viçosa: UFV. p. 817.
Pedrinho EAN (2009). Isolamento e caracterização de bactérias promotoras de crescimento em milho (*Zea mays* L.). Jaboticabal: Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho". p. 87.
Pereira LM, Pereira EM, Revolti LTM, Zingaretti SM, Moro GV (2015). Seed quality, chlorophyll content index and leaf nitrogen levels in maize inoculated with *Azospirillum brasilense*. Rev. Cienc. Agron. 46:630-637.
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. Appl. Microbiol. Biotechnol. 75:1143-1150.
Picazevicz AA, Kusdra JF, Moreno ADL (2017). Maize growth in response to *Azospirillum brasilense*, Rhizobium tropici, molybdenum and nitrogen. Rev. Bras. Eng. Agríc. Ambient. 21(9):623-627.
Portugal JR, Arf O, Peres AR, De Castilho Gitti D, Garcia NFS (2017). Coberturas vegetais, doses de nitrogênio e inoculação com *Azospirillum brasilense* em milho no Cerrado. Rev. Cienc. Agron. 48(4):639-649.
Portugal JR, Arf O, Peres AR, Gitti DC, Rodrigues RAF, Garcia NFS, Gare LM (2016). *Azospirillum brasilense* promotes increment in corn production. Afr. J. Agric. Res. 11:1688-1698.
Quadros PD (2009). Inoculação de *Azospirillum* spp. em sementes de genótipos de milho cultivados no Rio Grande do Sul. Porto Alegre: Universidade Federal do Rio Grande do Sul. p. 74.
Reis Júnior FB, Mendes IC, Reis VM, Hungria, M (2010). Fixação biológica de nitrogênio: uma revolução na agricultura. In. Faleiro FG, Andrade SRM, Reis Junior, FB (eds) Estado da arte e aplicações na agropecuária. Planaltina: Embrapa. pp. 247-281.
Reis Júnior FB, Reis VM, Silva LG, Döbereiner J (2000). Levantamento e quantificação de bactérias diazotróficas em diferentes genótipos de cana-de-açúcar (*Saccharum* spp.). Pesq. Agrop. Bras. 35:985-994.
Revolti LTM (2014). Interação genótipo vs formas de inoculação com *Azospirillum brasilense* em milho. Jaboticabal: Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agrárias e Veterinárias. 46 p.
Ritchie SW, Hanway JJ, Benson GO (1993). How a Corn Plant Develops, Special Report, n.48, Iowa State University of Science and Technology, Ames, Iowa.
Rodriguez H, Gonzalez T, Goire I, Bashan Y (2004). Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. Naturwissenschaften 91:552-555.
Rudnik P, Meletzus D, Green A, He L, Kennedy C (1997). Regulation of nitrogen fixation by ammonium in diazotrophic species of proteobacteria. Soil Biol. Biochem. 29:831-841.
Saikia SP, Bora D, Goswami A, Mudoi KD, Gogoi AA (2012). Review on the role of *Azospirillum* in the yield improvement of non-leguminous crops. Afr. J. Microbiol. Res. 6(6):1085-1102.
Sala VMR, Cardoso EJBN, Freitas JG, Silveira APD (2008). Novas bactérias diazotróficas endofíticas na cultura do trigo em interação com a adubação nitrogenada, no campo. Rev. Bras. Cienc. Solo. 32(3):1099-1106.
Silva AP, Arruda TF, Bach EE (2004). Ação do *Azopirillum brasilense* no desenvolvimento das plantas de trigo (variedade IAC-24) e cevada (variedade CEV 95033). Conscientiae Saúde 3:29-35.
Strzelczyk E, Kamper M, Li C (1994). Cytokinin-like-substances and ethylene production by *Azospirillum* in media with different carbon sources. Microbiol. Res. 149:55-60.
Swędrzyńska D, Sawicka A (2000). Effect of inoculation with *Azospirillum brasilense* on development and yielding of maize (Zea mays ssp. saccharata L.) under different cultivation conditions. Pol. J. Environ. Stud. 9(6):505-509.
Taiz L, Zeiger E (2013). Fisiologia vegetal. 5ed. Porto Alegre: Artmed,. 954 p.
Tien TM, Gaskins MH, Hubbell DH (1979). Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Appl. Environ. Microbiol. 37:1016-1024.
Travaglia C, Masciarelli O, Fortuna J, Marchetti G, Cardozo P, Lucero M, Zorza E, Luna V, Reinoso, H (2015). Towards sustainable maize production: Glyphosate detoxification by Azospirillum sp. and Pseudomonas sp. Crop Prot. 77:102-109.
United States Department of Agriculture (USDA) (2017). World corn supply and use. World agricultural supply and demand estimates. (Available online at https://www.usda.gov/). Access in oct 22, 2017.
United States Department of Agriculture (USDA) (2018). World corn supply and use. World agricultural supply and demand estimates. (Available online at https://www.usda.gov/oce/commodity/wasde/latest.pdf). Access in March 15, 2018.
Vazquez GH, Silva MRR, Sousa JFS, Baroles RD (2012). Fontes de *Azospirillum brasilense* e doses de nitrogênio em cobertura na cultura do milho. In. 29 Congresso nacional de milho e sorgo. Campinas: Instituto Agronômico/Associação Brasileira de Milho e Sorgo. pp. 1639-1645.
Verona DA, Duarte Junior JB, Rossol CD, Zoz T, Costa ACT (2010). Tratamento de Sementes de Milho com Zeavit®, Stimulate® e Inoculação com *Azospirillum* spp. In. Congresso Nacional de Milho e Sorgo, 18. Goiânia: Associação Brasileira de Milho e Sorgo.
Yang J, Kloepper JW, Ryu CM (2009). Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci. 14:1-4.