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Bibliographic Review

Agronomic performance of *Azospirillum brasilense* on wheat crops

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Abstract

The use of nitrogen-fixing diazotrophs bacteria in grasses is a recent alternative that which allows reducing the use of agricultural inputs. In this aspect the *Azospirillum brasilense* has emerged as a viable alternative in reducing nitrogen application in field crops. The objective of this review was to gather information regarding the agronomic performance in the use of *A. brasilense* in wheat crops. Besides the obvious reduction in the need for nitrogen fertilization, is noteworthy that the inoculation allows obtaining agronomic results similar to those obtained with fertilizers, that is, presents efficiency in several important agronomic attributes. Generally it is observed increase in productivity as the crop is inoculated. The best results were obtained with the use of diazotrophic bacteria associated to nitrogen fertilizer, underscoring the fact that the bacterium does not completely replace the fertilization. The increase in productivity is attributed to the improved translocation of biomass to grains and in the increased photosynthetic activity, justified by the increase in green tissues and its root system, occasioned by the plant symbiosis with *A. brasiliense*.

Keywords: biological nitrogen fixation; diazotrophic bacteria; nitrogen fertilization.

Desempenho agronômico de *Azospirillum brasilense* na cultura de trigo

Resumo

A utilização de bactérias diazotróficas fixadoras de nitrogênio em gramíneas é uma recente alternativa que permite diminuir o uso de insumos agrícolas. Neste aspecto a *Azospirillum brasilense* vem se destacando como uma alternativa viável na redução da aplicação de nitrogênio em culturas de lavoura. O objetivo desta revisão foi reunir informações com respeito ao desempenho agronômico na utilização de *A. brasilense* na cultura do trigo. Além da evidente redução na necessidade de adubação nitrogenada, é visível que a inoculação permite a obtenção de resultados agronômicos semelhantes aos obtidos com fertilizantes, ou seja, apresenta eficiência em várias das características agronômicas importantes. Geralmente observa-se elevação na produtividade quando a lavoura é inoculada. Pode-se verificar que os melhores resultados são relacionados a associação do uso da bactéria diazostrófica associado ao Nitrogênio, ressaltando o fato de que a bactéria não substitui completamente a adubação. O aumento de produtividade é atribuído à melhoria da translocação da biomassa até os grãos e no aumento da atividade fotossintética, justificado pela elevação dos tecidos verdes e aumento na eficiência do sistema radicular, ocasionado em razão da simbiose.

Palavras-chaves: fixação biológica de nitrogênio; bactéria diazotrófica; adubação nitrogenada.

Comportamiento agronómico de *Azospirillum brasilense* sobre el cultivo de trigo

Resumen

El uso de bacterias diazotróficas fijadoras de nitrógeno en pastos es una alternativa reciente que permite reducir el uso de insumos agrícolas. En este respecto el *Azospirillum brasilense* esta se destacando como alternativa viable para reducción de la aplicación de nitrógeno en los cultivos. El objetivo de esta revisión fue recopilar información sobre el comportamiento

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agronómico en el uso de *A. brasilense* en trigo. Además de la reducción obvia en la necesidad de fertilización con nitrógeno, es evidente que la inoculación agronómica permite la obtención de resultados similares a los obtenidos con los fertilizantes, es decir, presenta eficiencia en varias características agronómicas importantes. En general se observa aumento de la productividad cuando se inocula el cultivo. Se puede observar que los mejores resultados se relacionan con el uso de bacterias diazostrófica asociados con el nitrógeno, lo que subraya el hecho de que la bacteria no reemplaza completamente los fertilizantes. El aumento de la productividad se atribuye a la mejora de la translocación de la biomasa hasta el grano y el aumento de la actividad fotosintética, justificado por el aumento en los tejidos verdes y de la eficiencia del sistema radicular, ocasionado por la simbiosis.

Palabras clave: fijación biológica de nitrógeno; bacterias diazotróficas; fertilización nitrogenada

Introduction

In Brazil, the main problems faced in agriculture are due to the disorderly advance of the agricultural frontier, to the inadequate use of crop rotation systems and to the lack of harnessing of the prior cultivation residues, besides of the low efficiency of many fertilizers that which are increasing the production cost (REIS, 2007).

According to COSTA et al. (2006), among the used fertilizers, the nitrogen stands out for being one of the essential nutrients for maintaining productivity in grasses, mainly acting in the proteins, chloroplasts formation and in the synthesis of compounds which constitutes the vegetal structure, in this way, relating to the plant height, size of the stem and general characteristics of grains.

For being a nutrient of high demand, the nitrogen fertilizer represents the greatest cost among the fertilizers, besides of the fact that the intensive use of chemical and organic nitrogen fertilization tends to generate an environmental problem, the water and soil contamination with nitrates (EIRAS and COELHO, 2011).

However, as reported by MOREIRA et al. (2010), actually there are alternatives which allow increasing the efficiency of inputs use, being verified a great interest in alternative practices that aim the inputs application reduction, one of them is the atmospheric nitrogen fixation (BNF) done by bacteria denominated diazotrophic, which posses the capacity of being a versatile nutritional organism, consuming a variety of organic compounds which are found freely in the rhizosphere and consequently becoming assimilable to the plants.

Throughout time, the study with the use of bacteria, which are promoters of growth, has appeared in the perspective of assisting the improvement of the nitrogen nutrient mechanism (BASHAN and DE-BASHAN, 2005). RADWAT et al. (2004) verified that the endophytic diazotrophic bacteria have shown, through many studies, a contribution in the plant nutrition, both through biological fixation of atmospheric nitrogen as in the production of phytohormones that acts in the plants root system, what can result in better absorption of minerals and water.

REIS JÚNIOR et al. (2002) highlight that the group *Azospirillum* possess seven studied diazotrophic species, being them *A. brasilense, A. lipoferum, A. amazonense, A. irakense, A. halopraeferans, A. largimobile* and *A. dobereinera*. Researches inform that the specie *A. brasilense* has presented satisfactory results in agroecosystems when associated to plants of the Poaceae family, such as the maize, oat and wheat (DÖBEREINER, 1992).

The use of a combination of strains of the *Azospirillum* bacterium, associated to small doses of nitrogen has shown to be more efficient when compared to isolates of the bacterium. The efficiency found in many researches is between 60% and 70% (OKON, LABANDERA-GONZALES, 1994; REIS, 2007).

As stated by REIS JÚNIOR et al. (2002), studies focused on the use of the *A. brasilense* are found in many cultures, in special in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) and occasionally in other poaceas, like the rice (*Oryza sativa* L.) and the white oat (*Avena sativa* L.). In relation to the tropical forage species, are found studies referent to the use of *A. lipoferum*, *A. amazonense* and *A. brasilense* associated to *Brachiaria* spp., *Digitaria decumbens* and *Pennisetum purpureum.*

Among the studied winter grasses for the use of *A. brasilense*, the wheat stands out for its economical importance. In Brazil, during the season 2011/2012 the planted area was 2,166.2 ha, with a yield of 2,672 kg ha^{-1}, totalizing a production of 5,788.6 tons (CONAB, 2012). In this sense, the use of this bacterium can assist in the potential phytotechnical increase of these crops, as well as reduce the costs of nitrogen inputs used by the same.

This research has as objective to approach scientific studies related to the use of *A. brasilense* and its relation in the agronomical development of wheat crop (*Triticum aestivum* L.).

Efficiency in yield

Regarding the influence of the *A. brasilense* in the wheat crop yield, the use of these bacteria tends to provide efficiency in translocation of the plant biomass to the grains, making it heavier and more full (DIDONET et al., 2000). Among the strains with good efficiency on wheat, stand out the Ab-V1, Ab-V5, V6 and Ab-Ab-V8, showing a production increase varying from 312 to 423 kg ha^{-1}, i. e. an increase between 13% to 18%, being that the strains Ab-V5 and V6 presented a potential increase of up to 31% (HUNGRIA et al., 2010). In a general context, the inoculation can entail a yield increase between 10 to 30%, however, few information present high increments when situated in values between 50 to 250% (BASHAN and LEVANONY, 1990; STRAVOS and GEORGE, 2010).

MENDES et al. (2011) found results that demonstrate the use of the bacteria as efficient on the wheat crop, being the best result achieved with the use of 110 kg of N + 300 mL ha^{-1} of *A. brasilense* producing 2,957 kg ha^{-1}, followed by 2,939 kg ha^{-1} when using 55 kg ha^{-1} of N associated to 100 mL ha^{-1} of *A. brasilense*. In this sense, the use of this bacterium reduced in 50% the need of using nitrogen fertilizers for results significantly similar. While the treatment without inoculants produced 2,873 kg ha^{-1} in the dose of 110 kg of N ha^{-1}, and 2,485 in the dose of 55 kg of N ha^{-1}, only this last one differing significantly, such results emphasize the yield increase with the use of inoculants.

Tests with the use of *A. brasilense* RAM-7 presented a better yield in the wheat grains, generating an increase of 7.4% in comparison to the non use of *A. brasilense.* Treatments using doses of 100% of nitrogen demanded by the crop, associated to the bacteria, generated an increase of 9% in the production, in relation to the dose of 100% of N without the bacteria (DALLA SANTA et al., 2004), being these data similar to the found by REYNDERS and VLASSAK (1982), where the use of the strains S631 and SpBr14 added an increase of 9.14% and 14.82%, respectively, in the agricultural yield.

DIDONET et al. (1996) in their studies, concluded that the use of inoculants in the form of strains of peat moss powder JA04 with 15 kg ha^{-1} of N in the wheat sowing, completely substitutes the nitrogen fertilizer of 15 kg ha^{-1} of N in the sowing and 45 kg ha^{-1} in cover, without the use of the diazotrophic bacteria. This result proves that the inoculants can substitute the cover fertilization without generating losses in grains yield. The same author reports that only the use of *A. brasilense* strain of peat moss powder JA04 without N fertilization produced approximately 29.1% more in relation to the non use of N and inoculants in the control treatment.

SALA et al. (2007) reported that the inoculation with the diazotrophic bacterium IAC-AT-8 of *A. brasilense* promoted an increase in the wheat yield when associated to increasing doses of nitrogen fertilizer, with maximum yield of 3,905 kg ha^{-1} at the dose of 120 kg ha^{-1} of N. These data are in agreement with those of MILLET and FELDMAN (1984) and COROSSA et al. (2013), who justify that the grains yield increase is due to the addition of nitrogen fertilizer with the bacterium, because it causes increase in the grains number per ear and in the number of ears per plant.

DALLA SANTA et al. (2008) obtained results in the wheat crop which indicate the inoculation efficiency due to the increase of 23.9% in the grains yield through inoculation with *A. brasilense* RAM-7 and without nitrogen fertilization in relation to the treatment without inoculation and nitrogen. This author also reports that the use of inoculants with 48 kg ha^{-1} of N showed statistically similar results to treatments with 60 kg ha^{-1} of N, with or without inoculation.

Tests done in 297 locations in the Pampas of Argentina during the seasons 2002 – 2006 verified that was an average increase of 260 kg ha^{-1} in the wheat yield with the use of liquid inoculants, being that in 70% of these locations there was a higher yield with the bacteria use. The inoculants use, associated to the recommended dose of N for the wheat crop, generated a production average of 3,900 kg ha^{-1} and 4,160 kg ha^{-1} respectively, resulting in an increase of 8% in yield, with the *A. brasilense* application (DÍAZ-ZORITA and FERNÁNDEZ-CANIGIA, 2008).

According to BALDANI et al. (1987), the inoculation of wheat seeds with *A. brasilense*, strain 245, produced around 31% more than the control, in grains yield, yet, these data were not statistically significant in relation to the non inoculation of seeds.

However, CAMPOS et al. (1999) assessing the *A. brasilense* use in areas with remains of maize, without any fertilizer quantity obtained a production

of 1,228 kg ha^{-1} in the wheat crop, being this inferior to the control without inoculants (1,399 kg ha^{-1}). The best result in the maize remains area was with the inoculants use associated to 15 kg of N ha^{-1} in the sowing and 45 kg of N ha^{-1} in the cover, with yield average of 1,833 kg ha^{-1}. In areas with beans remains, the highest production was of 2,389 kg ha^{-1} with the same treatment. In locations with turnip remains, the best result was obtained with inoculants associated to 15 kg of N ha^{-1} in the sowing and without cover, producing 3,598 kg ha^{-1}, having no statistical difference with the use of 15 kg de N ha^{-1} in the sowing and 45 kg de N ha^{-1} in cover and without the use of the inoculant bacteria.

Influence in the Nitrogen content in the plant and grains

Regarding the influence of the bacteria on wheat seeds germination, few studies show viability on the use of this technique. RAMPIM et al. (2012) and GRANADO et al. (2012) verified that there was no statistical difference with the use of the *A. brasiliense* in the three wheat genotypes.

Relating the N contents present in the wheat crop (plant and grains), DIDONET et al. (1996) found meaningful results in the treatments with strains of *A. brasilense* in the period of physiological maturation of the crop, highlighting the use of peat moss powder, strain 245, as the higher accumulator of N in the wheat plants aerial parts during the maturation. According to the same author, twenty days after the anthesis, the total nitrogen accumulated in the plant was only superior due to a result of inoculation.

As stated by JEZEMWSKI et al. (2010) the wheat inoculation with *A. brasilense* provided a greater extraction of N and accumulation in the grains, which were incremented by addition of a growth regulator, the smaller grains weight were found in treatments without inoculants and growth regulator, being that the grains weight increase is related to a rise of availability of N during the reproductive stage of the plant.

SALA et al. (2008) found results in the wheat crop tillering, which shows that the isolate IAC-AT-8 of *A. brasilense* along with the dose of 60 and 120 kg ha^{-1} of N, provided significant increase in biomass and increment of the N accumulated in the plant, in relation to the treatments with inoculants and without N, and without both. These same authors state that the N content in the grains and the mass of thousand seeds did not have influence of the inoculation, therefore, the yield was determined by the number of grains and not by their mass.

Regarding the influence of this bacteria in relation to the N content in the wheat grains, BALDANI et al. (1987), detected greater accumulation percentage of this element in the treatments with the strains of *A. brasilense* associated to nitrogen doses of 60 to 100 kg of N ha^{-1}, such results are in agreement with those of RODRIGUES et al. (2000), who evidenced significant growth of the N content in the grain, in the treatments with the bacteria use, without the addition of N.

Tests done with wheat in area with maize and soybean remains demonstrate that the treatments differed regarding the total N content in the grain, being the results influenced only by the quantity of nitrogen fertilization, independent if it was being used or not the bacterium *A. brasilense*, the more explicit differences were in the areas with maize remains, highlighting that the treatments where there was increase in N dose presented a higher N content in the grains (CAMPOS et al., 1999).

DIDONET et al. (2000) affirm that the inoculation with the strain of *A. brasilense* 245 provided better harnessing of the N accumulated in the biomass, translocating more efficiently it to the grains, thus producing heavier grains and with greater volume. The same study also indicated a small quantity of N present in the plants straw, by occasion of the physiological maturation in the inoculated treatments, probably due to the better relocation of the plant to the grains in these treatments.

As for the hectoliter weight of the wheat grains, the use of 110 kg ha^{-1} of N associated to the use of *A. brasilense* in the dosages of 100 mL/150 kg and 100 mL/50 kg of seed, obtained respectively, 75.8 kg hL^{-1} and74.4 kg hL^{-1}, being that these significantly differed from the other treatments where were used only 50% of the nitrogen fertilization and also in the control without inoculants, which obtained 70.2 kg/hL, being this the smaller value. Therefore, it was verified an improvement in the hectoliter weight, being that it was positively affected by the use of *A. brasilense* via seeds treatment, independent of the used dose (MENDES et al., 2011). These data collaborate with those from CORASSA et al. (2013), where the use of inoculants with N in cover substitutes the N applied in the base, not modifying the hectoliter weight in the wheat grains.

The best yield results by the use of inoculation with *A. brasilense* are shown in Table 1.

Table 1. Best results obtained with treatment of *A. brasilense*.

Wheat crop		
Authors	**Treatments**	**Yield (kg ha^{-1})**
Dalla Santa *et al*. (2008)	60 kg ha^{-1} of N	2,420
Mendes *et al*. (2011)	110 kg ha^{-1} of N + 300 mL of *A. brasilense*	2,957
Didonet *et al*. (1996)	15 kg of N ha^{-1} in the sowing	3,520
Campos *et al*. (1999)	15 kg in the sowing	3,765
	15 kg ha^{-1} in the sowing + 30 kg ha^{-1} in the cover[2]	2,389

[1] *Done in areas with turnip remains.*

[2] *Done in areas with beans remains.*

Accumulation of Nitrogen in green and dry mass

Verifying the green and dry mass of the wheat crop, some experiments presented a significant increase of N at the grain milky-ripe stage, for the doses of 40 kg of N ha^{-1} (KAPULNIK et al., 1987).

During the tillering, the use of *A. brasilense*, with increasing addition of N assisted the increase of the production of dry mass in wheat, having no statistical difference between the doses 60 and 120 kg of N ha^{-1} and being superior to the treatment without inoculation and nitrogen fertilization (SALA et al., 2008). As stated by the same authors, there was increase in the accumulated N in the ear by the use of *A. brasilense.*

In this sense, according to similar studies of GRANADO et al. (2012), the treatments with the use of strains of this bacterium did not only increase the content of green and dry mass, as well as raised the contents of stem weight and increase in the chlorophylls quantity when compared to the control. SAUBIDET et al. (2002), states that the inoculated plants presented a greater production of biomass, thus assisting in the plant growth and in the roots absorption of nutrients.

However, DIDONET et al. (2000) affirms that there was no statistical difference in the wheat dry mass accumulation with or without inoculation, both at the anthesis stage as well as in the maturation. Yet, it is noteworthy that in the anthesis the application of the strain 245 of *A. brasilense* and isolate 10 of *A. lipoferum* together, affected the ears weight in relation to different doses and seasons of nitrogen application, however it did not occur in stem and leaves, thus, it was verified that the accumulation of biomass up to the period of anthesis was smaller when it was used smaller doses of N in the sowing.

Such variations can be justified, as reported by studies done by DOBBELAERE et al. (2002), where these found variations according to the concentrations of the inoculum, being that low concentrations (10^5 – 10^6 ufc plant^{-1}) stimulated the development of the dry matter and roots, while the high concentrations (10^7 – 10^8 ufc plant^{-1}) did not present any results related to dry matter. In the root system, high concentrations inhibited the growth.

Effects in the root system

With relation to the use of *A. brasilense* in the development of wheat plants, the inoculation presents effects regarding the roots, causing not only alterations in the morphology of the root system and in the rhizoid increase, but also in the diameter of the lateral, primary and secondary roots. It is due to the elongation in the same, what causes an increase in the root and plant size, and thus, generates an increase in the contact surface for the retrieving of water and nutrients for the plant, besides of assisting in the nitrogen fixation (SILVA et al., 2004).

BASÃ and LEVANONY (1988) observed that after the inoculation of the *A. brasilense*, these bacteria were strongly adsorbed by the soil and then migrated to the plant, when reached the roots, formed small aggregates in the root surface and an internal population inside the cortex, besides of others connected to the cortical and epidermal walls. In this sense, the bacteria induced in the increase of the index of protrusions extrusions of the roots, besides of the increase in the root tip cellular division.

EGORENKOVA et al. (2000), analyzing the initial stages of the cells colonization of the *A. brasilense* in wheat roots, verified that the adsorption of strains by the roots is greater in the first hours after the infection, then it passes through a stabilization

stage. As occurs an increase in the incubation period, the bacteria fixation in the roots becomes more intense, however, the fixation of bacterial cells by the roots depends of the strain and of the concentration that is going to be used, such as in the case of the mutant strain Sp245 Gri^+, which present the smallest capacity of adsorption (SHELUD´KO et al., 2010) in the concentrations 10^8 and 10^9 ufc $plant^{-1}$, tending to inhibit the roots development.

DIDONET et al. (2000) found results showing that the application of smaller doses of N generated smaller indexes of accumulated dry mass in the wheat, this can be a result of a greater investment of the plants in their roots system, instead of the aerial part, this can also occur by the use of inoculation. According to the same authors, many studies have shown that infected plants with bacteria of the genus *Azospirillum* promote a meaningful increase in the biomass and root volume, in special in the initial stages of plants development.

ROESCH et al. (2005) concluded that the inoculated plants with the diazotrophic bacteria had its root length affected, presenting a greater efficiency and achieving an increment of 2.3 times in relation to the average of plants without the use of inoculants, besides of the fact that the bacteria were capable of fixating N_2 and transfer it to the plants *in vitro*, supplying its needs until the 21 days after the germination.

Thus, it is verified that the use of diazotrophic bacteria, which belong to the genus *Azospirillum*, promote results that emphasize not only the gains in biologically fixating the nitrogen, as well as assist in a greater extension of its root system, consequently increasing the area of the soil exploitation (OKON e VANDERLEYDEN, 1997). The increase of the area in the root system in inoculated plants assists in a greater longevity in the green tissues. In this sense, occurs an increase in the photosynthetic capacity, what consequently provides a greater translocation and increase in the weight and accumulated N in the grains (TERMAN, 1979).

However, it is clear that such satisfactory results are obtained in adequate environmental conditions. FISCHER et al. (2000) analyzing the colonization of these bacteria in many environments, verified that the plants under salt stress presented colonization only in the roots tip, contrary to the control in normal environment, which presented wide distribution in the other root parts. This fact shows that the efficacy of the bacteria use is directly linked with the environmental conditions present in the planting location.

Conclusions

The use of the bacteria *A. brasilense* presents promising results in the plants development and in the wheat grains yield. However, is necessary more detailed studies referent to the mechanisms which result in improvements in the final productivity, i. e. citing the mechanisms of translocation of the plant nitrogen to the grains and the relation of the bacteria with the root growth, besides of analyzing a quantity of diazotrophic bacteria or dosage that is efficient for the success of the same. Yet, it can be verified an increase tendency in the root system of the infected plants, increasing the accumulated N content in the grains and partial substitution of nitrogen fertilization due to improvement in the efficiency of the same, generated by the inoculation, being this factor affected not only by it, but by its relation with the remains of the prior crop, stage of N application, cultivar used, strain used, combined use with growth regulators, among other factors.

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