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EFFECT OF NITROGEN-FIXING BACTERIA ON GRAIN YIELD AND DEVELOPMENT OF FLOODED IRRIGATED RICE[1]

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ABSTRACT - This study aimed at evaluating the effect of *Azospirillum brasilense*, a nitrogen-fixing bacterium, on flooded irrigated rice yield. Evaluations were carried out in a shaded nursery, with seedlings grown on an Alfisol. Were performed two sets of experiments. In the first, were carried out four experiments using the flooded rice cultivars INIA Olimar, Puitá Inta-CL, Br Irga 409 and Irga 424; these trials were set up as completely randomized design in a 5x4 factorial scheme, with four replications. Treatments consisted of five nitrogen rates (0, 40, 80, 120 and 160 kg ha^{-1}) and four levels of liquid inoculant Ab-V5 and Ab-V6 - *A. brasilense* (0, 1, 2 and 4 times the manufacturer's recommendation) without seed treatment. In second set, were performed two experiments using the cultivars Puitá Inta-CL and Br Irga 409, arranged in the same design, but using a 4x2 factorial. In this set, treatments were composed of four levels of Ab-V5 and Ab-V6 - *A. brasilense* liquid inoculant (0, 1, 2 and 4 times the recommendation of 100 mL ha^{-1}), using rice seeds with and without insecticide and fungicide treatment. Shoot dry matter, number of panicles, and rice grain yield per pot were the assessed variables. The results showed that rice seed inoculation with *A. brasilense* had no effects on rice grain yield of the cultivars INIA Olimar, Puitá Inta-CL, Br Irga 409 and Irga 424.

Keywords: *Azospirillum brasilense*. Grain yield. *Oryza sativa.*

BACTÉRIA FIXADORA DE NITROGÊNIO E PRODUTIVIDADE E DESENVOLVIMENTO DE ARROZ IRRIGADO POR INUNDAÇÃO

RESUMO - O uso de bactérias fixadoras de nitrogênio do gênero *Azospirillum brasilense* tem sido recomendado para gramíneas como milho, trigo e arroz, porém seus benefícios necessitam ser melhor estudados. O objetivo deste trabalho foi avaliar o efeito da bactéria fixadora de nitrogênio *A. brasilense* na produtividade de arroz irrigado por inundação. O experimento foi conduzido em viveiro agrícola revestido com sombrite, utilizando um Plintossolo Háplico. No primeiro conjunto de experimentos utilizaram-se as cultivares de arroz irrigado por inundação INIA Olimar, Puitá Inta-CL, Br Irga 409 e Irga 424, constituindo 4 experimentos em delineamento experimental inteiramente casualizado, em esquema fatorial 5 x 4, com quatro repetições. Os tratamentos foram constituídos por 5 doses de nitrogênio (0, 40, 80, 120 e 160 kg ha^{-1} de N), e 4 doses de inoculante líquido composto por bactérias *A. brasilense* cepas Ab-V5 e Ab-V6 (0, 1, 2 e 4 vezes a recomendação). No segundo conjunto de experimentos, com as cultivares Puitá Inta-CL e Br Irga 409, utilizou-se o esquema fatorial 4 x 2. Quatro doses de A. brasilense cepas Ab-V5 e Ab-V6 (0, 1, 2 e 4 vezes a recomendação de 100 mL ha^{-1}), sem e com tratamento das sementes do arroz com inseticidas e fungicidas. Foram avaliados a massa da matéria seca da parte aérea, número de panículas e produtividade de grãos de arroz/vaso. A inoculação das sementes de arroz irrigado por inundação, cultivares INIA Olimar, Puitá Inta-CL, Br Irga 409 e Irga 424, com *A. brasilense* cepas Ab-V5 e Ab-V6 não aumenta a produtividade de grãos de arroz.

Palavras-chave: *Azospirillum brasilense*. rendimento de grãos. *Oryza sativa*.

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INTRODUCTION

The symbiosis between leguminous plants and nitrogen-fixing bacteria, so-called rhizobia, brings significant contributions for biological nitrogen fixation (HUNGRIA et al., 2007). This process is believed to meet part of the nitrogen demand of grasses and may be performed by bacteria of various genera such as *Herbaspirillum*, *Burkholderia* and *Azospirillum*, which have been isolated from plants like rice, wheat, corn and sorghum (HUNGRIA, 2011). These bacteria have diverse N-fixing mechanisms in grasses, many of them are free-living organisms (OKON; LABANDERA-GONZALES, 1994), being bacteria and plants species-specific and strongly affected by the environment (nitrogen and oxygen availability, and presence of other microorganisms). Among the studied bacterial species, *Azospirillum brasilense* can be highlighted because of its outstanding performance when associated with grass plants, such as corn and wheat (HUNGIA et al., 2010; NOVAKOWISKI et al., 2011; PICCININ et al., 2013).

Even though inoculation with bacteria have brought significant results in grain yield of grasses, these outcomes are not very consistent due to the lack of further study (HUNGRIA et al., 2010; HUNGRIA, 2011; MENDES et al., 2011; MÜLLER et al., 2016). Yield substantial increases have been observed in corn and wheat crops with the use of certain *A. brasilense* bacterial strains (HUNGRIA et al., 2010; MÜLLER et al., 2016), which are commercial lineages registered by the Brazilian Department of Agriculture (HUNGRIA, 2011), being available for corn, wheat and rice.

The *Azospirillum* genre is widely spread and can be found at varied population densities depending on its interaction with the used cultivar and the environment. In flooded environments, as in flooded rice farming, these microaerophilic bacteria find a favorable environment, improving crop nutrition and development (SOUZA et al., 2000). These bacteria occur in rice fields colonizing roots and stems endophytically (SILVA et al., 2004). Once there is no formation of apparently specialized structures for N_2 fixation (nodules), and these microorganisms might invade plant tissue through wounds, cracks caused by lateral root emergence, mechanical injuries, and stomata, spreading throughout the plant by sap vessels (REIS et al., 2006).

Different responses can be achieved by inoculating *Azospirillum* strains with wheat, rice, corn and sorghum, and average grain yield ranges between 5 to 30% (FERREIRA et al., 2003; HUNGRIA et al., 2010; MÜLLER et al., 2016). However, there are few studies in flooded rice fields, due to a limited number of strains tested with this crop and low availability of commercial inoculant. In producing regions from western Rio Grande do Sul state, in Brazil, rice seeds have been inoculated with bacteria since the 2011/ 2012 harvest season; however, it is still poorly understood and documented the interaction between inoculants and nitrogen fertilizer, rice cultivars, management systems, as well as further grain yield gains.

The objective of this study was evaluate the effect of inoculating *A. brasilense*, a nitrogen-fixing bacterium, on flooded irrigated rice development and grain yield.

MATERIAL AND METHODS

Were performed two sets of experiments, totaling six trials. They were carried out in a shaded nursery during the 2012/ 13 harvest season, in the city of Itaqui-RS, Brazil (29º 12’ 28’’ South, 56º 18’ 28’’ West and 64 m altitude), using an Alfisol (EMBRAPA, 2013). The soil was sampled within a depth of 0-20 cm and subsequently sieved through 4 mm mesh for flooded rice cultivation. According to chemical analysis, the soil characteristics were pH H_2O= 5.1; P= 12.6 mg dm^{-3} K= 0.153; Ca= 2.7; Mg= 0.7; Al= 0.6 cmol$_c$ dm^{-3} V= 50%; OM= 1.6%. Soil liming was performed three months before sowing, as recommended by SOSBAI (2014).

In the first set of experiments, were conducted trials using four cultivars of flooded rice (INIA Olimar, Puitá Inta-CL, Br Irga 409 and Irga 424). For these, were applied a completely randomized design in a 5x4 factorial scheme, with four replicates in 7.5 L pots (6 L soil/ pot). Treatments consisted of five nitrogen rates (0, 40, 80, 120 and 160 kg ha^{-1}) and four levels of liquid inoculant strains Ab-V5 and Ab-V6 - *Azospirillum brasilense* (0, 1, 2 and 4 times the manufacturer's recommendation - Masterfix). The recommendation for N application is 120 kg ha^{-1} (SOSBAI, 2014); while for the inoculant is 100 mL ha^{-1}, under a concentration of 2 x 10^8 colony forming units/ mL. The manufacturer recommends this concentration of inoculant for corn and rice.

In the second set of experiments, were conducted trials using two flooded rice cultivars (Puitá Inta-CL and Br Irga 409). Both trials were performed in completely randomized design and a 4x2 factorial scheme, with four replicates. The treatments consisted of four levels of liquid inoculant strains Ab-V5 and Ab-V6 - *A. brasilense* (0, 1, 2 and 4 times the manufacturer's recommendation), and using treated and untreated seeds. Seeds were treated using the following insecticides Fipronil (Standak® 80 mL 100 kg^{-1} of seed) and Imidacloprid (Gaucho® 67 mL 100 kg^{-1} of seed), and the fungicides Carboxin + Thiram (Vitavax Thiram 200 SC® 200 mL 100 kg^{-1} of seed).

At sowing, fertilization was applied at a rate of 10 kg ha^{-1} N, 350 kg ha^{-1} P (triple superphosphate), and 300 kg ha^{-1} K (potassium chloride). The P and K doses were five times the field recommendation; fertilizers were milled in Wiley mill and homogenized with all soil in pot. Seeds were inoculated with *A. brasilense* strains on October 17, 2012, and then allowed to shade dry for 15 min, before being sown. Six seeds were sown by pot at a depth of 3 cm. At 14 days after sowing (DAS), each pot were thinned, leaving two equally spaced plants. The first 50% of the total amount of N was top dressed at 20 DAS, when plants reached V3/ V4 stage (COUNCE et al., 2000). The next day, pots were filled with water up to 4 cm above soil surface, being kept constant until harvest. At panicle differentiation stage - R0 (40 DAS), the remaining 50% of N was applied as urea. After emergence and throughout cropping, pots' locations were exchanged every two times by week to provide similar light conditions for all plants (treatments and replications).

Harvest was carried in February of 2013, about 120 DAS, with a difference of few days among cultivars. Hereinafter, we evaluated shoot dry matter, panicle number and rice grain yields per pot.

The results were submitted to F test (variance analysis) and the means compared by the Tukey test for qualitative factors; and for quantitative ones, data underwent regression analysis.

RESULTS AND DISCUSSION

Figure 1 shows that the cultivars INIA Olimar, Puitá Inta-CL, Br Irga 409 and Irga 424 showed similar results regarding shoot dry matter, panicle number and rice grain yield in response to N levels and strains of *A. brasilense* (Ab-V5 and Ab-V6).

For the four cultivars, all assessed variables increased up to the rate of 160 kg ha^{-1} N, and grain yield presented a quadratic increasing trend in the four cultivars (Figure 1A). This might have occurred by the low OM content in soil (1.6%) and by the supply of proper amounts of P and K, maximizing the production potential of the crop to utilize the applied N. According Genro Jr. et al. (2010), once high amounts of P, K are supplied to rice crops, a maximum N-use efficiency can be achieved in both irrigated and dry seeding systems, mainly under high technological level and suitable climatic conditions.

There was no interaction between N levels (0 to 160 kg.ha^{-1}) and *A. brasilense* bacterial strains (Ab-V5 and Ab-V6). Therefore, none of the rice cultivars responded in production to any of the doses applied of *A. brasilense* strains (Figure 1B).

Grain production has not increased with inoculation. A possible incompatibility between strains and rice cultivars may have affected this response. Despite being found records at the Ministry of Agriculture, Livestock and Supply (MAPA) for upland rice, wheat and corn, there is no specific record for flooded rice. In this context, Campos et al. (2003) reported that certain rice genotypes had different responses with respect to biological N_2 fixation, and some of them have achieved positive responses. Similarly, Moura (2010), who evaluated BRS Primavera cultivar inoculated with Ab-V5 and Ab-V6 strains, did not observed any grain yield increase. Moreover, it is known that *A. brasilense* 'Sp245' enhanced root and shoot growth of rice seedlings for most upland rice cultivars (EMBRAPA, 2003). Furthermore, *Herbaspirillum seropedicae* are able to increase rice grain yield in up to 50%, depending on the strain used (GUIMARÃES et al., 2003).

Similar results to these found in this study were reported by Campos et al. (2000) for inoculation with the commercial product Graminante® (*A. brasilense*), which is recommended for corn. This inoculant did not promote any grain yield gains as well; and the authors attributed that to a lack of specificity of the bacteria with corn hybrids (CAMPOS et al., 2000).

According Roesch et al. (2007), the interaction between diazotrophs and corn hybrids to fix nitrogen or promote plant growth depend on many biotic and environmental factors, such as cultivar, soil microbial community and N availability. Following that principle, we provided N low and high doses to the soil; however, positive results on *A. brasilense* inoculation were not confirmed. This way, we can state that another factor, other than N, was responsible for the lack of bacterial growth and rice yield gain. We suggest that an absence of symbiosis between rice cultivars and *A. brasilense* strains Ab-V5 and Ab-V6.

Silva et al. (2004) and Cardoso et al. (2010) observed that *Azospirillum* spp. bacteria could develop at satisfactory densities in irrigated rice, under similar weather conditions, colonizing endophytically roots and stems. These authors also checked that soil chemical properties had no effect on the symbiont, except for N fertilizations that may reduce its population. Thus, the association can contribute to improve crop nutrition and growth (SOUZA et al., 2000). Therefore, it was shown that bacteria are of major importance to biological N_2 fixation; though, further research is required to select strains with greater affinity for these rice cultivars, since Ab-V5 and Ab-V6 have not presented successful results.

Differently in wheat cropping, inoculations using these strains, Ab-V5 and Ab-V6, increased grain yield, increasing savings on N mineral fertilizers (HUNGRIA et al., 2010; PICCININ et al., 2013). In corn, there were also obtained grain yield increments using the strains Ab-V5 and Ab-V6 (HUNGRIA et al., 2010; BRACINI et al., 2012; MÜLLER et al., 2016).

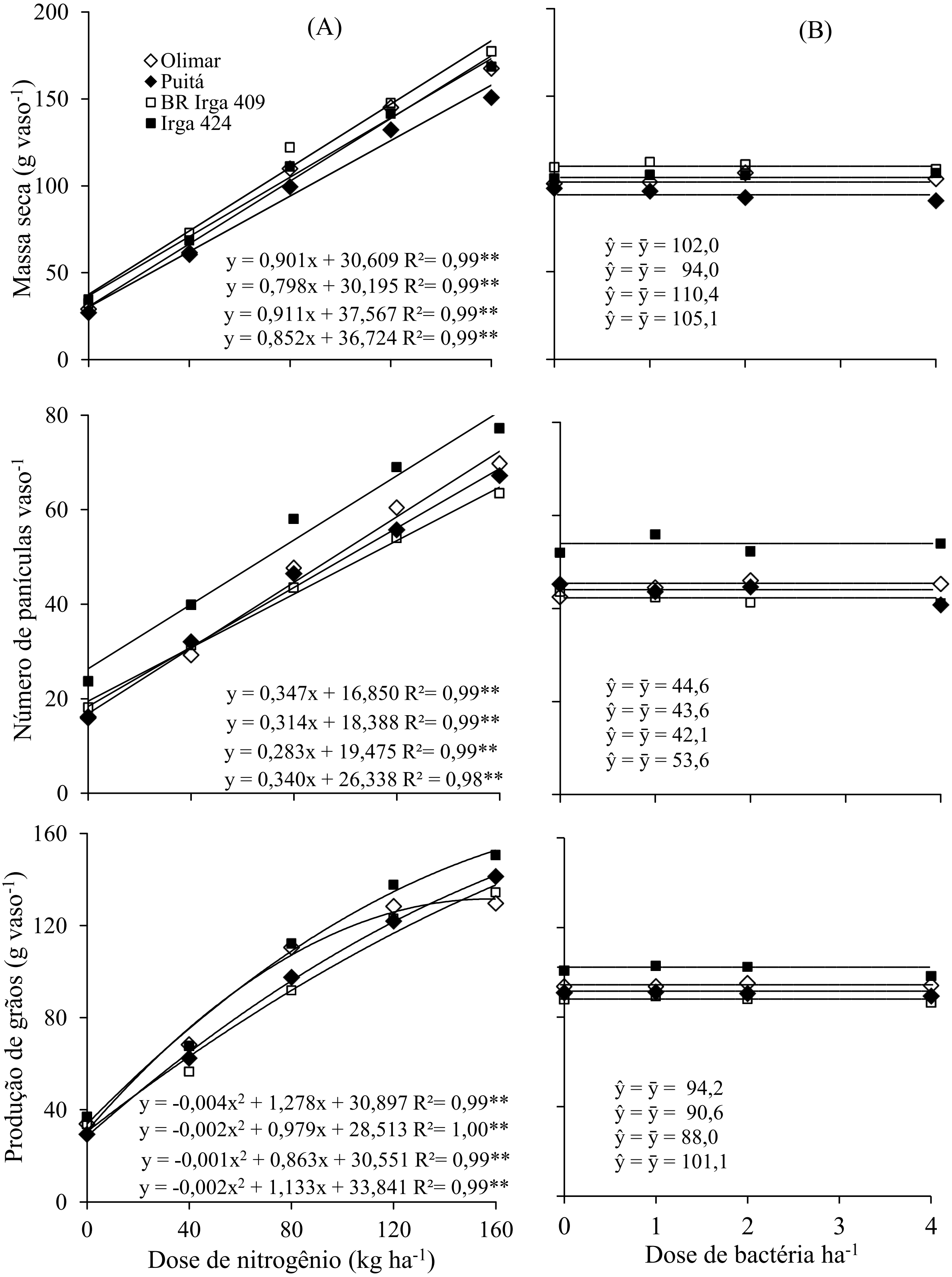

Figure 1. Mass of the dry matter of aerial part, number of panicle and flooded rice grain yield, cultivars Olimar, Puitá Inta-CL, Br Irga 409 e Irga 424, in relation the N level (A) and *A. brasilense* level (B). ** Significant at 1%.

Treating seed with insecticides and fungicides has not altered the response of rice cultivars to inoculation with the strains used, as can be seen in Tables 1 and 2, and Figure 2. Despite increasing dry matter and number of panicles, seed treatment did not increase grain yield for the cultivars Putiá Inta CL-i and Br IRGA 409. That fact is because treated seeds generate rice seedlings protected from insect and fungi infestations in the early crop cycle (SOSBAI, 2012). Nonetheless, this protection does not necessarily ensure increased yield, since other factors are involved in that variable, such as soil fertility, water supply, management system, besides temperature and sunlight conditions.

Therefore, *A. brasilense* strains Abv5 and Abv6 (Masterfix®) had no effect on increasing yields of flooded rice, unlike what has been reported in other crops as wheat and corn (HUNGRIA, 2011; MÜLLER et al., 2016), for which the same commercial product is also recommended.

Table 1. Influence of bacteria *Azospirillum brasilense* in mass of the dry matter of aerial part, number of panicle and grain yield of flooded rice in cultivars Puitá Inta-CL e Br Irga 409, without N application, with seeds treated and not treated with insecticides and fungicides.

Treatment*	Dry mass (g)	Number of panicle	Grain yield (g)
	Puitá Inta-CL		
Without seed treatment	27.1 b	16.2 b	29.5 a
With seed treatment	34.3 a	20.9 a	32.2 a
	Br Irga 409		
Without seed treatment	34.3 b	18.2 b	33.8 a
With seed treatment	40.9 a	20.5 a	33.6 a

Averages followed whit the same letter, in column and in the same cultivar, not differ at 5% of probability of error.

Table 2. Analysis of variance to flooded rice grain yield/pot, cultivars Puitá Inta-CL e Br Irga 409, whereas two experiments in randomized factorial scheme 4 x 2 (four levels of bacteria, whit and whitout seed treatment).

Causes of variation	Degree of Freedom	Sum of squares	Medium Square	F
		Puitá Inta-CL		
Factor A (bacteria)	3	90.99	30.33	2.11 ^{ns}
Factor B (T.seed)	1	57.67	57.67	4.02 ^{ns}
Factor A x B	3	46.13	15.38	1.07 ^{ns}
		Br Irga 409		
Factor A (bacteria)	3	59.81	19.94	1.09 ^{ns}
Factor B (T.seed)	1	0.53	0.53	0.03 ^{ns}
Factor A x B	3	149.61	49.87	2.73 ^{ns}

^{ns} not significant.

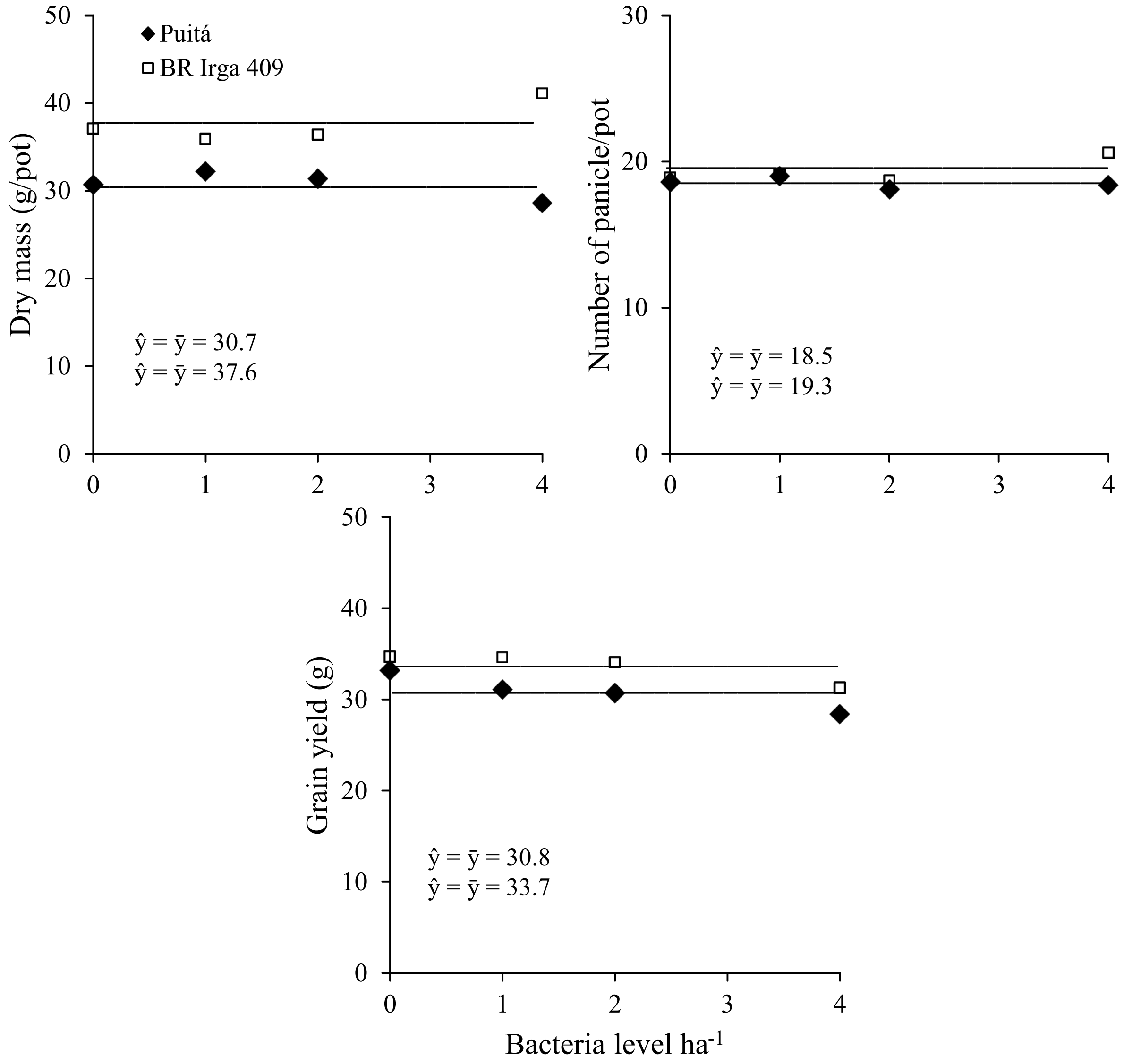

Figure 2. Mass of the dry matter of aerial part, number of panicle and flooded rice grain yield/pot (two plants), cultivars Puitá Inta-CL e Br Irga 409, in relatction of *A. brasilense* level In absence of mineral N.

CONCLUSION

Inoculation of the flooded irrigated rice cultivars INIA Olimar, Puitá Inta-CL, Br Irga 409 and Irga 424 with *Azospirillum brasilense* strains Ab -V5 and Ab-V6 (Masterfix®) have no significant effect on rice crop yields.

Rice seeds treated with insecticide and fungicide (Fipronil and Imidacloprid, and Carboxin + Thiram) have no influence on the performance of both *A. brasilense* strains (Ab-V5 and Ab-V6) to increase shoot dry matter, panicle number, and rice grain yields.

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