

Growth Promotion by *Azospirillum brasilense* in the Germination of Rice, Oat, Brachiaria and Quinoa

ABSTRACT

Aims: The inoculation with growth promoting bacteria in cultivated crops is an alternative to reduce the use of chemical compounds minimizing environmental damage and production costs. According to outstanding benefits associated with low researches with the crops in question, the objective of the present work was to evaluate the characteristics of the development of *Oryza sativa*, *Avena sativa*, *Brachiaria 75 ruziziensis* and *Chenopodium quinoa* inoculated with growth promoting bacteria *Azospirillum brasilense*.

Study design: The experimental design was completely randomized in a 4 x 4 factorial scheme with four replicates of 50 seeds.

Place and Duration of Study: The experiment was conducted in a greenhouse, located at the Experimental Station of Horticulture and protected cultivation of the State University of Western Paraná at Marechal Candido Rondon city, 54° 22' W and 24° 46' S, and altitude of 420 m.

Methodology: At greenhouse, the species were inoculated with *Azospirillum brasilense* then sowed in autoclaved sand forming the following treatments: no inoculation, seed inoculation, foliar inoculation, foliar and seed inoculation. At seven days after sowing, the germination percentage was evaluated and at 21 days after emergence the morphological characteristics of the crop.

Results: The inoculation with bacteria favored seed germination of quinoa. However, for the crops of vetch and brachiaria, the statistic results of fresh weight accumulation were superior when inoculated. Some of the evaluated variables demonstrated possible negative effects of the inoculation, but these may be associated to the crops being sowed in an inert material, without the presence of necessary nutrients to plant development, such as nitrogen, which is an essential factor to obtain good results with the *A. brasilense* application.

Conclusion: For the quinoa culture the seed inoculation favored germination, increasing it by 17%, a result considered promising, even if the differences were not maintained throughout the seedling development.

Keywords: Plant growth promoting bacteria; Inoculation; Establishment of the seedling.

1. INTRODUCTION

In order to increase production and reduce costs, with less environmental damages, the use of inoculant stands out, especially with *A. brasilense* bacterium that promotes the growth of plants, found in Brazilian soils, with good adaptability for many crops, besides its production is easy for commercial use [1].

The adaptability of *A. brasilense* to colonize many species is proven throughout the years of research. In rice, Hahn *et al.* [2], analyzing the initial development variables and the efficiency of the inoculation, emphasize that the presence of the bacterium promoted the initial growth of the seedling, besides the efficient root colonization and increased root number and mass of the inoculated plants. In addition, Gitti *et al.* [3], working with upland rice, associated with different plant coverage, nitrogen doses and bacterial inoculation, highlights that, in association with plant cover, inoculation with *A. brasilense* added significant benefits and variables for crop productivity.

Ortega and Salamone [4], studying the bacterial community of oats and rye, emphasize that the intensive use of agrochemicals drastically affects the natural microbiota of cultivated plants, reducing yield variables and that inoculation with growth promoting bacteria can help to remedy these damages and restore the natural microbiota. In research on inoculated grasses, it is emphasized that the use of growth promoting bacteria in oats, wheat and barley can be considered as a partial substitute of nitrogen fertilization [5].

Inoculation with *Azospirillum* spp. in crops such as maize, wheat, sorghum and sugarcane are studied over a longer period and have shown significant and positive results in relation to growth promotion [6]. However, some crops with significant endophytic potential did not receive great research highlights, losing prominence when compared to those with the more cultivated area.

Studying inoculation with *A. brasilense* on *Brachiaria* spp., Hungria, Nogueira and Araújo [7], highlight the potential of bacteria in the establishment and recovery of degraded pastures, in addition to contributing with their accumulation of nutrient. Hanisch, Baldinot Junior and Vogt [8], in their pasture researches, comment on the importance of studying how nitrogen fertilization can negatively influence the action of *A. brasilense* inoculation, thus ensuring consistent results.

The quinoa culture has immense potential for endophytic colonization by growth-promoting bacteria. On studies in the western region of Paraná, evaluating the growth of quinoa plants in soils originating from diverse sources were obtained 130 bacterial colonies with endophytic potential [9]. Quinoa seeds inoculated with *Bacillus* spp., showed lower germination time and greater germination uniformity, as well as morphological variables, such as height, root length and stem diameter, higher than those non-inoculated [10].

As *A. brasilense* benefits from plant development when associated with some crops, there is an interest in knowing if these benefits can extend to other crops of economic interest.

According to outstanding benefits associated with low researches with the crops in question, the objective of the present work was to evaluate the characteristics of the development of *Oryza sativa*, *Avena sativa*, *Brachiaria 75 ruziziensis* and *Chenopodium quinoa* inoculated with growth promoting bacteria *Azospirillum brasilense*.

2. MATERIAL AND METHODS

The experiment was carried out in a greenhouse, located at the Experimental Station of Horticulture and protected cultivation "Prof. Dr. Mario César Lopes" of State University of Western Paraná in the city of Marechal Cândido Rondon, 54°22'W, 24°46'S and altitude of 420 m.

Seeds of rice (*Oryza sativa*), oats (*Avena sativa*) and brachiaria (*Brachiaria ruziziensis*) were obtained from local producers in the city of Marechal Candido Rondon, Paraná. The quinoa

(*Chenopodium quinoa* var. Q318) seeds were obtained from the quinoa breeding research group of State University of Western Paraná, Marechal Cândido Rondon. The seeds were sown in 5-liter trays completely filled with previously autoclaved sand at 105°C and 1 atm for 2 hours, being the sowing depths of 0,5 cm for brachiaria and quinoa, and 1,0 cm for rice and oats.

At the sowing day, the seeds were aseptized in hypochlorite and 70% alcohol, then the seed inoculation was done with commercial inoculant containing the plant growth promoting bacteria (PGPB) *Azospirillum brasilense*, strains Abv5 and Abv6, in the concentration of 2.0×10^8 viable cells per ml for all crops. Leaves were inoculated seven days after sowing, the concentration used was obtained by rule of three, based on the concentration of 100 ml ha⁻¹ of inoculant according to the recommendation of the manufacturer, with a hand sprayer.

The experimental design was completely randomized in a 4 x 4 factorial scheme, with four replicates and each replicate had 50 seeds. The first factor is the species - rice, oats, brachiaria and quinoa - and the second one refers to inoculation forms of *A. brasilense*: control (without inoculation), seed inoculation, foliar inoculation and seed and foliar inoculation. Each tray was divided into four sessions and each session received a replicate of a randomized treatment.

In the control treatment, the seeds did not receive inoculant application. For seed inoculation, they were immersed directly in the product for 30 minutes. In the treatment of foliar inoculation, the plants received the application of the inoculants aerially and in the inoculation of seeds plus the foliar inoculation the product was applied in both forms. The trays were wetted until the waterlogging and then taken to the greenhouse. The moisture was visually checked twice a day, every day until the evaluations, being added more water if necessary.

Seven days after sowing, percentage of germination was evaluated. After 21 days were evaluated the following parameters: seedlings initial development, number of leaves, measurement of the root and aerial part length with a graduated ruler, as well as root and aerial part fresh and dry weight, being the dry weight accrued after the plants passed 24 hours in a forced air circulation oven at 65°C.

The data were submitted to analysis of variance, and when significant compared by the Tukey test with 5% probability of error, using the SISVAR software [11].

3. RESULTS AND DISCUSSION

Table 1 shows the results obtained for percentage of germination, the average number of leaves, aerial and root length according to the evaluated crops and the different forms of inoculation with *A. brasilense*, where it was observed a significant difference between treatments for the interaction.

Regarding germination, the highest percentages were verified when quinoa was inoculated only on the seed (up to 17% increase), whereas there was no significant statistical difference for the other species. This result was similar to those found by Borges *et al.* [12] in tests with temperature variation, where quinoa seed germination varied between 74 and 87%.

127 **Table 1. Percentage of germination, number of leaves, aerial height and root length of**
128 **rice, oats, brachial and quinoa, submitted to different forms of inoculation with *A.***
129 ***brasiliense* (Abv5 and Abv6)**

	Control	Seed	Foliar	Seed+foliar	Average
Percentage of germination (%)					
Rice	89,00*	90,50	88,50	86,00	88,50
Oats	83,60	94,30	83,20	93,90	88,75
Brachiaria	42,00	38,00	38,50	37,50	39,00
Quinoa	69,00 b	80,50 ab	70,00 b	86,50 a	76,50
Average	70,88	75,75	70,13	76,00	73,19
Number of leaves					
Rice	2,76	2,83	2,74	2,88	2,80
Oats	3,15 ab	2,78 b	3,52 a	2,95 b	3,10
Brachiaria	2,72	2,73	2,83	2,41	2,67
Quinoa	5,43	5,55	5,05	5,16	5,30
Average	3,51	3,47	3,53	3,35	3,47
Aerial length (cm)					
Rice	10,83	10,17	11,15	10,59	10,68
Oats	7,63 a	7,80 a	6,14 b	7,66 a	7,31
Brachiaria	4,50	4,13	4,04	4,07	4,18
Quinoa	3,44	3,46	3,51	3,50	3,48
Average	6,60	6,39	6,21	6,45	6,41
Root length (cm)					
Rice	13,99	11,68	10,38	14,03	12,52
Oats	16,82 a	13,15 ab	12,28 b	13,76 ab	14,00
Brachiaria	20,31 a	19,04 a	20,58 a	12,93 b	18,21
Quinoa	3,34	3,66	2,73	3,91	3,41
Average	13,61 a	11,88 ab	11,49 ab	11,16 b	12,04

130 *Averages followed by the same capital letter in the column and the same lower-case letter in the row,
131 do not differ by Tukey test, ($p \leq 0,05$).

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133 This positive result can be explained because *A. brasiliense* helps to release hormones, such
134 as auxins and gibberellins, which induce the embryogenic process due to the overcoming of
135 quiescence. The bacteria penetrate the tegument together with the entry of water, enhancing
136 root growth and increasing the absorption potential of water, resulting in a stimulus to the
137 development of the plant [13]. Moreira *et al.* [14] complement that in the case of grasses
138 from the genus *Brachiaria* the penetration is difficult due to the tegument being more
139 impermeable, a factor that may have interfered in the germination of the seeds of some
140 species studied.

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142 In addition, it is important to mention that seed inoculation has a variable influence on
143 germination and early seedling development, either because of the species or cultivar [15].
144 Evaluating the application of *A. brasiliense* on grasses, Vogel and Fey [16] verified that the
145 bacteria inoculation assisted in the initial process of germination of rye, but the effect was
146 not pronounced until the end of the process resembling the control; while in the triticale crop,

the percentage of germination was higher than the control at the end of the process. These results, together with those found in this study, demonstrate that different crops may present variable inoculation responses in their development.

It is also observed that the average numbers of oat leaves were smaller than the control in both treatments with foliar application; as well as lower oat aerial length results when foliar application of *A. brasilense* was performed, as well as root length results significantly lower than control when foliar inoculation was carried out in oats and seed and leaf inoculation in brachiaria; while the other treatments with inoculation resemble the control for the mentioned parameters. Similarly, studies by Sipione *et al.* [17] with *X Triticosecale wittmack* indicated that the use of foliar inoculation reduced plant height and, in general, the use of *A. brasilense* did not result in alterations in the initial growth of the species.

The responses obtained with inoculation may be influenced by several mechanisms and abiotic factors [18]. Evaluating wheat performance, Piccinin *et al.* [19] observed that inoculation with *A. brasilense* is dependent on nitrogen fertilization. Alves, Francisco and Carvalho [20] complement that the inoculation was favorable when together with the nitrogen until determined dose. However, in the present study, sowing was performed in inert material, without nutrients, which may have interfered in this relation, resulting in negative effects on the crop development, or even in the absence of positive effects in response to inoculation with *A. brasilense*.

Regarding the aerial part fresh and dry weight there was no difference for the interaction or for the averages of the treatments isolated in each factor, for the species in question. The roots fresh and dry weight presented significant interaction between the factors under study (Table 2).

Table 2. Aerial part and root fresh and dry weight of rice, oats, brachiaria and quinoa, submitted to different way of inoculation with *A. brasilense* (Abv5 and Abv6)

	Control	Seed	Foliar	Seed+foliar	Average
<i>Aerial part fresh weight (g)</i>					
Rice	0,0574*	0,0589	0,0637	0,0544	0,0586
Oats	0,0776	0,0695	0,0652	0,0682	0,0701
Brachiaria	0,0402	0,0359	0,0395	0,0320	0,0369
Quinoa	0,0271	0,0320	0,0206	0,0230	0,0257
Average	0,0506	0,0491	0,0472	0,0444	0,0478
<i>Aerial part dry weight (g)</i>					
Rice	0,0144	0,0145	0,0148	0,0138	0,014
Oats	0,0137	0,0128	0,0121	0,0126	0,013
Brachiaria	0,0082	0,0075	0,0084	0,0085	0,008
Quinoa	0,0043	0,0048	0,0040	0,0038	0,004
Average	0,0102	0,0099	0,0098	0,0097	0,0099
<i>Root fresh weight (g)</i>					
Rice	0,2086 a	0,1932 ab	0,1685 ab	0,1611 b	0,1829
Oats	0,2140 a	0,1626 b	0,1420 b	0,1710 ab	0,1724
Brachiaria	0,1025	0,0843	0,1096	0,0993	0,0989
Quinoa	0,0202	0,0204	0,0240	0,0367	0,0253

Average	0,1363 a	0,1151 ab	0,1110 b	0,1170 ab	0,1199
Root dry weight (g)					
Rice	0,0494	0,0469	0,0344	0,0438	0,0436
Oats	0,0277 b	0,0443 a	0,0326 ab	0,0277 b	0,0331
Brachiaria	0,0162 b	0,0172 b	0,0262 ab	0,0395 a	0,0248
Quinoa	0,0082	0,0134	0,0072	0,0174	0,0116
Average	0,0254	0,0305	0,0251	0,0321	0,0283

*Averages followed by the same capital letter in the column and the same lower-case letter in the row, do not differ by Tukey test, ($p \leq 0,05$).

The fresh root weight of rice was significantly lower when inoculated with seeds and leaves, and the other treatments with *A. brasilense* resembled for both this treatment and the control. In the oats, smaller results were found with the seed application and with the foliar performed separately, that is, better results were obtained with the control and the application of inoculation of the two forms together (although this also resembled the treatments separately).

Meanwhile, root dry weight presented alterations between treatments, different from those obtained for fresh weight. In oats, larger masses were observed with seed inoculation and foliar inoculation separately; while for brachiaria, better results were obtained with foliar and foliar+seed application, positively differing from control in both species.

It is important to mention that the establishment of seedling can be characterized as the phase in which the external conditions begin to become competent to carry out the photosynthesis and to assimilate the water and nutrients of the soil [21]. In this sense, due to the experiment being carried out in an inert substrate, without the addition of nutrients, it is possible that the initial seedling development was affected, increasing the water content in some treatments (related to the larger amounts of the fresh weight) without effective nutrient absorption and dry mass increase, as occurred for rice and oat control treatments.

On the other hand, for oats and brachiaria roots dry weight, it was observed that the treatments with inoculation of *A. brasilense* favors the fixation in larger quantities when compared to control. Dartora *et al.* [22], analyzing the effect of inoculation on the initial development of grasses highlights emphasizes an increase in the mass of the roots of inoculated plants when compared to the control.

4. CONCLUSION

For the quinoa culture the seed inoculation with *A. brasilense* favored germination, increasing it by 17%, a result considered promising, even though the differences were not maintained throughout the development of the seedling.

Some of the evaluated parameters demonstrated possible negative effects of the inoculation, but these may be associated with the sowing of the crops in inert material, without the presence of nutrients necessary to plant development, such as nitrogen, which is an essential factor to obtain satisfactory results with the *A. brasilense* application.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.



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Figura 1. Morfologia radicular de culturas inoculadas com *Azospirillum brasilense* em microscópio ótico em aumento de 10 vezes. A – Aveia, controle, B – Aveia, inoculação via semente, C – Aveia, inoculação via foliar, D – Aveia, inoculação via semente e foliar, E – Arroz, controle, F – Arroz, inoculação via semente, G – Arroz, inoculação via foliar, H – Arroz, inoculação via semente e foliar, I – Braquiária, controle, J – Braquiária, inoculação via semente, K – Braquiária, inoculação via foliar, L – Braquiária, inoculação via semente e foliar, M – Quinoa, controle, N – Quinoa, inoculação via semente, O – Quinoa, inoculação via foliar, e P – Quinoa, inoculação via semente e foliar.

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Figura 2. Imagens da morfologia das plantas cultivadas em areia submetidas a inoculação com *Azospirillum brasilense*, divididos por tratamentos: ausência de inoculação (T1), inoculação via semente (T2), inoculação via foliar (T3), inoculação via semente e foliar (T4).

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