

Original Research Article

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 reducing environmental damage and production costs. The objective of this research was to evaluate the growth promotion of the bacteria *Azospirillum brasilense* in the initial development of *Oryza sativa*, *Avena sativa*, *Brachiaria ruziziensis* and *Chenopodium quinoa*.

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 State University of Western Paraná at Marechal Cândido Rondon city, 54° 22' W and 24° 46' S, and altitude of 420 m.

Methodology: At greenhouse, the species were inoculated *Azospirillum brasilense* then seeded in autoclaved sand forming the following treatments: no inoculation, seed inoculation, foliar inoculation, foliar and seed inoculation. At seven days after they were seeded 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 parameters demonstrated possible negative effects of the inoculation, but these may be associated to the crops sowing in 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. The bacterial inoculation in oat and brachiaria seeds promoted the accumulation of fresh weight.

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

1. INTRODUCTION

Plant growth promoting bacteria *Azospirillum brasilense* was discovered on the 70's and is one of the bacteria that most arouses interest in researchers, acting as an important contributor to plant nutrition participating in the biological fixation of nitrogen, inducing the phytohormones production which act in different phases of plant development, solubilizing compound, among other factors that allow better water and mineral salts absorption and utilization by plants [1].

Aiming for increased production and cost reduction with less environmental damage, the use of inoculant stands out especially with *A. brasilense*, a bacterium that promotes plant growth, found in Brazilian soils, with a good adaptability to many crops, besides the ease of production for commercial use [2].

The increase of nitrogen fixation and growth promotion, from the interaction crop-bacteria, may depends on biotic factors, as plant genotype, and abiotic factors, such as soil microbiology and nitrogen available [3].

The adaptability of *Azospirillum brasilense* to colonize many species is proven throughout the years of research. In rice, Hahn *et al.* [4], analyzing initial development parameters and inoculation efficiency, emphasize that the presence of the bacterium promoted the initial growth of the seedling studied, besides presenting efficient root colonization and increased root number and mass in the inoculated plants. Also, Gitti *et al.* [5], 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 parameters for crop productivity.

Ortega e Salamone [6], 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 parameters, and that inoculation with growth promoting bacteria can help to remedy these already significant damages, and reestablish the natural microbiota. Research on inoculated grasses emphasizes that the use of growth promoting bacteria in oats, wheat and barley can be considered as a partial substitute for nitrogen fertilization [7].

Inoculation with *Azospirillum* spp. in crops such as corn, wheat, sorghum and sugarcane are studied over a longer period and have shown significant positive results in relation to growth promotion [8]. However, some crops with significant endophytic potential have not received great research highlights, losing prominence when compared to those with more cultivated area.

Studying inoculation with *A. brasilense* on *Brachiaria* spp., Hungria, Nogueira and Araújo [9], highlight the potential of bacteria in the establishment and recovery of degraded pastures, in addition to contribute with their nutrient accumulation. Hanisch, Baldinot Junior and Vogt [10], in their pasture researches, comment on the importance of studying how nitrogen inoculation can negatively influence the action of *A. brasilense*, thus ensuring consistent results.

The quinoa culture has immense potential for endophytic colonization. Studies in the western region of Paraná, evaluating quinoa plants growth in soils originated from different sources, obtained 130 bacterial colonies with endophytic potential [11]. Quinoa seeds inoculated with *Bacillus* spp., showed lower germination time and higher germination uniformity, as well as morphological parameters, such as height, root length and stem diameter, higher than those non-inoculated [12].

Because the *A. brasilense* benefits from vegetal development when associated with some crops, there is an interest in whether 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 growth promotion of *Azospirillum*

brasiliense bacterium in the initial development of *Oryza sativa*, *Avena sativa*, *Brachiaria ruziziensis* e *Chenopodium quinoa*.

2. MATERIAL AND METHODS

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

The rice (*Oryza sativa*), oats (*Avena sativa*) and brachiaria (*Brachiaria ruziziensis*) seeds were obtained from local producers of Marechal Candido Rondon city, Paraná. The quinoa (*Chenopodium quinoa* var. Q318) seeds were obtained from the research group in quinoa breeding of State University of Western Paraná, Marechal Cândido Rondon.

The experimental design was completely randomized in a 4 x 4 factorial scheme with four replicates of 50 seeds. The first factor is the species – rice, oats, brachiaria and quinoa – and the second one referred to *A. brasiliense* inoculation: control, seed inoculation, foliar inoculation and seed and leaf inoculation.

At the sowing day, seeds were asepsis with hypochlorite and 70% alcohol, after the seed were inoculated using commercial inoculant containing the plant growth promoter (PBVG) *Azospirillum brasiliense*, strains Abv5 and Abv6, in the concentration of 2,0 x 10⁸ viable cells per ml. At 7 days after emergence, foliar inoculation was realized using the concentration of 100 ml. ha⁻¹ of inoculants, with a hand sprayer.

The sowing was carried out on October 23, 2017 in sand substrate, previously autoclaved at 105 °C, 1,5 atmosphere for 30 minutes, in a depth of 0,5 cm for brachiaria and quinoa, and 1 cm for rice and oats.

Seven days after sowing, were evaluated the seedlings percentage of germination. After 21 days were evaluated the seedlings initial development, regarding the root and aerial part length with a graduated ruler, as well as root and aerial part fresh and dry weight (forced air circulation oven at 65°C). Also, was analyzed morphological parameter root development, using optical microscope with increase of 10X.

The data were submitted to variance analysis, and when significant compared by the Tukey test with 5% probability of error, using the SISVAR software [13].

3. RESULTS AND DISCUSSION

Table 1 shows the results obtained for percentage of germination, average number of leaves, shoot height and root length in relation to the evaluated cultures and inoculation ways of *A. brasiliense*, were it was observed the there was a significant difference between treatments for the interaction.

Table 1. Percentage of germination, average number of leaves, aerial height and root length from rice, oats, brachiaria and quinoa, submitted to the treatments of control (C), seed inoculation (S), foliar inoculation (F) and both inoculation (S + L).

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

*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, a 5% probability.

Regarding germination, the best percentages were verified when the quinoa was inoculated only on the seed (up to 17% increase), whereas there was no significant statistical difference for the other species. The results were similar to those found by Borges *et al.* [14] in tests with temperature variation, whose germination varied between 74 and 87%.

This positive result can be explained because *A. brasilense* helps to release hormones, such as auxins and gibberellins, which induce the embryogenic process due to the overcoming of quiescence [15].

In addition, it is important to mention that seed inoculation has a variable influence on germination and early seedling development, whether due to the species or cultivar [16]. Evaluating the application of *A. brasilense* on grasses, Vogel and Fey [17] verified that the bacteria inoculation assisted in the initial process of germination of rye, but the effect was 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 varying 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 height 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.

Responses obtained with inoculation can 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.

Regarding the aerial part fresh and dry weight there was no statistical difference for interaction or for the treatments averages applied to the cultures. The root fresh and dry weight presented statistical difference for the interaction between the factors (Table 2).

Table 2. Aerial part and root fresh and dry weight of rice, oats, brachiaria and quinoa, submitted to treatments control (C), seed inoculation (S), foliar inoculation (F), both inoculation (S + F).

	Control	Seed	Foliar	Seed+foliar	Average
<i>Aeria part fresh weight (g)</i>					
Rice	0,0574*	0,0589	0,0637	0,0544	0,0586 B
Oats	0,0776	0,0695	0,0652	0,0682	0,0701 A
Brachiaria	0,0402	0,0359	0,0395	0,0320	0,0369 C
Quinoa	0,0271	0,0320	0,0206	0,0230	0,0257 D
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 A
Oats	0,0137	0,0128	0,0121	0,0126	0,013 B
Brachiaria	0,0082	0,0075	0,0084	0,0085	0,008 C
Quinoa	0,0043	0,0048	0,0040	0,0038	0,004 D
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 A
Oats	0,2140 a	0,1626 b	0,1420 b	0,1710 ab	0,1724 A
Brachiaria	0,1025	0,0843	0,1096	0,0993	0,0989 B
Quinoa	0,0202	0,0204	0,0240	0,0367	0,0253 C
Average	0,1363 a	0,1151 ab	0,1110 b	0,1170 ab	0,1199
<i>Root dry weight (g)</i>					

Rice	0,0494	0,0469	0,0344	0,0438	0,0436 A
Oats	0,0277 b	0,0443 a	0,0326 ab	0,0277 b	0,0331 B
Brachiaria	0,0162 b	0,0172 b	0,0262 ab	0,0395 a	0,0248 C
Quinoa	0,0082	0,0134	0,0072	0,0174	0,0116 D
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, a 5% probability.

Fresh root weight of rice was significantly lower when inoculated via seed and leaf, and the other treatments with *A. brasilense* resembled both this treatment and the control. In the oats, smaller results were found with the seed application and with the foliar route 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 seedling establishment can be characterized as the phase in which the external conditions begins to become competent to carry out the photosynthesis and assimilate water and nutrients from the soil [21]. In this sense, due to the experiment Being carried out in an inert substrate, without the presence 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 fixation and dry mass increase, as occurred for rice and oat control treatments.

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

For all analyzed variables, statistical differences were verified in relation to the crops, which can be justified by the genetic and specific characteristics of each species.

4. 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.

The bacterial inoculation in oat and brachiaria seeds promoted the accumulation of fresh weight.

Finally, some of the evaluated parameters demonstrated possible negative effects of the inoculation, but these may be associated to the crops sowing in 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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