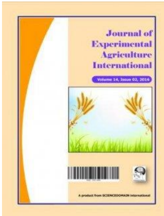

Journal of Experimental Agriculture International

22(1): 1-9, 2018; Article no.JEAI.39729
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

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

Tauane Santos Brito[1*], Daniele Cristina Schons[1], Giovana Ritter[1], Leila Alves Netto[1], Tatiane Eberling[1], Renan Pan[1] and Vandeir Francisco Guimarães[1]

[1]Agraian Sciences Center, West of Paraná State University, Pernambuco Street, 1777, 85960-000, Marechal Cândido Rondon, P.R. Brasil.

Authors' contributions

This work was carried out in collaboration between all authors. Authors TSB and DCS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GR, LAN, TE and RP managed the analyses of the study. Authors TSB and DCS managed the literature searches. Author VFG reviewed and corrected all mistakes. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2018/39729
Editor(s):
(1) Masayuki Fujita, Professor, Dept. of Plant Sciences, Faculty of Agriculture, Kagawa University, Japan.
Reviewers:
(1) Necat Toğay, Mugla S. K. University, Fethiye ASMK Vocational High School, Turkey.
(2) Stefan Martyniuk, IUNG-PIB Pulawy, Poland.
(3) Tariq Mukhtar, Pir Mehr Ali Shah Arid Agriculture University, Pakistan.
Complete Peer review History: http://www.sciencedomain.org/review-history/23949

Original Research Article

Received 11th January 2018
Accepted 19th March 2018
Published 2nd April 2018

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, 54º22' W and 24º46' S, and altitude of 420 m.

**Corresponding author: E-mail: tauane-brito@hotmail.com, tauane-brito@hotmai.com;*

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 showed possible negative effects of 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 proven throughout the years research. In rice, Hahn et al. [2] analyzing the initial development variables and the inoculation efficiency, emphasize that the presence of the bacterium promoted the seedling initial growth 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 rice, emphasize that 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 with 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. MATERIALS AND METHODS

The experiment was carried out in a greenhouse, located at Experimental Station of Horticulture and protected cultivation "Prof. Dr. Mario César Lopes" State University of Western Paraná in the city of Marechal Cândido Rondon, 54º22'W, 24º46'S and altitude 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 105ºC 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 asepsis 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 are the species - rice, oats, brachial 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 inoculant application aerially and in the inoculation of seeds plus foliar inoculation the product was applied in both forms. The trays were wetted until 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 65ºC.

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 different forms of inoculation with *A. brasilense*, were 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%.

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. The bacteria penetrate the tegument together with the entry of water, enhancing root growth and increasing the absorption potential of water, resulting in a stimulus to the development of the plant [13]. Moreira et al. [14] complement that in the case of grasses from the genus *Brachiaria* the penetration is difficult due to the tegument being more impermeable, a factor that may have interfered in the seeds germination on some species studied.

Table 1. Percentage of germination, number of leaves, aerial height and root length of rice, oats, brachial and quinoa, submitted to different forms of inoculation with *A. brasilense* (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

**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,($p \leq 0{,}05$)*

In addition, it is important to mention that seed inoculation has a variable influence on germination and early seedling development, either because of the species or cultivar [15]. Evaluating the application of *A. brasilense* on grasses, Vogel and Fey [16] 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 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 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 other treatments with inoculation resemble the control for the mentioned parameters. Similarly, studies by Sipione et al. [17] with *X Triticosecale wittmack* indicated that use foliar inoculation reduced plant height and, in general, the use of *A. brasilense* did not result in alterations in 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 interaction or 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).

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 on 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 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 from 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. brasiliense* favors the fixation in larger quantities when compared to control. Dartora et al. [22], analyzing the effect of inoculation on initial development of grasses highlights emphasizes an increase in the mass of the roots of inoculated plants when compared to control.

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
			Aerial part fresh weight (g)		
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
			Aerial part dry weight (g)		
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
			Root fresh weight (g)		
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 ≤0,05)*

Fig. 1. The morphology of plants cultivated in sand submitted to inoculation with *Azospirillum brasilense*, divided by treatments: no inoculation (T1), seed inoculation (T2), foliar inoculation (T3), seed and foliar inoculation (T4)

Fig. 2. Root morphology of cultures inoculated with *Azospirillum brasilense* in a 10-fold magnification optical microscope. A - Oat, control; B - Oat, seed inoculation; C - Oat, foliar inoculation; D - Oat, inoculation via seed and foliar; E - Rice, control; F - Rice, inoculation via seed; G - inoculation via foliar; H - Rice, inoculation via seed and foliar; I - Brachiaria, control; J - Brachiaria, seed inoculation; K - Brachiaria, foliar inoculation; L - Brachiaria, inoculation via seed and foliar, control; N - Quinoa, seed inoculation; O - Quinoa, foliar inoculation; and P - Quinoa, seed and leaf inoculation

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 seedling development. .

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Coelho LF. Freitas SS, Melo AMT, Ambrosano GMB. Interaction of fluorescent *Pseudomonas* and *Bacillus* spp. with distinct plant rhizospheres. Revista Brasileira de Ciência do Solo, Portuguese. 2007;31:1413-1420.
2. Hahn L, De Sá ELS, Filho BDO, Machado RG, Raquel Garibaldi Damasceno RD, Giongo A. Rhizobial inoculation, alone or coinoculated with *Azospirillum brasilense*, promotes growth of wetland rice. Revista Brasileira de Ciência da Solo. 2016;40:1-15,.
3. Gitti DC, Arf O, Portugal JR, Corsini DCDC, Rodrigues RAF, Kaneko FH. Cover crops, nitrogen rates and seeds inoculation with *Azospirillum brasilense* in upland rice under no-tillage. Bragantia. Portuguese. 2012;4(71):509-517.
4. Ortega JSE, Salamone IEG. Effect of fertilization and combined inoculation with *Azospirillum brasilense* and *Pseudomonas fluorescens* on rhizosphere microbial communities of Avena sativa (Oats) and Secale Cereale (Rye) grown as cover crops. International Journal of Agricultural and Biosystems Engineering. 2017;11(4)1.
5. Santa ORD, Hernández RF, Alvarez GLM, Junior PR, Soccol CR. Influence of *Azospirillum* sp. inoculation in wheat, barley and oats. Ambiência. 2008;4(2): 197-207.
6. Hungria M, Nogueira MA, Araujo RS. Inoculation of *Brachiaria* spp. with the plant growth-promoting bacterium *Azospirillum brasilense*: An environment-friendly component in the reclamation of degraded pastures in the tropics. Agriculture, Ecosystems and Environment. 2016;221: 125-131.
7. Hungria M, Campo RJ, Souza EM, Pedrosa FO. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. Plant and Soil. 2010;331(1/2):413-425.
8. Hanisch AL, Baldinot Júnior AA, Vogt GA. Productive performance of *Urochloa brizantha* cv. Marandu as a function of inoculation with *Azospirillum* and nitrogen doses. Revista Agro@mbiente On-line. 2017;11(3):200-2008.
9. Andrioli KK. Occurrence of endophytic bacteria associated to quinoa with potential to promote plant growth. 2016;71. Dissertation (Master in Agronomy)– Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon; 2016. Portuguese.
10. Machaca MYL. Bacterias solubilizadores de fosfato del género Bacillus em suelos de la província de el collao (Puno) y su effecto en la germinación y crescimiento de quinua (*Chenopodium quinoa* Willd.) en condiciones de invernadero. 2017. 89p. Dissertação (Mestrado em Microbiología y Labatorio Clínico) – Universidad Nacional del Altiplano, Puno, Spanish; 2017.
11. Ferreira DF. Sisvar: A guide for its bootstrap procedures in multiple comparisons. Science e Agrotechnology, Portuguese. 2014;38:109-112.
12. Borges CT, Costa CJ, Soares VN, Meneghello GE, Gadotti GI, Villela FA, Deuner C, Troyjack C. Germination of quinoa seeds submitted to different temperatures. Research and Development Bulletin. Portuguese. 2016;245:1-18.
13. Cassán F, Perriga D, Sgroya V, Masciarellia O, Pennab C, Lunaa V. *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). European Journal of Soil Biology. 2009;45(1):28-35.
14. Moreira CDA, Pereira DH, Coimbra RA, Moreira IDA. Germination of grasses due to inoculation diazotrophic bacteria. Scientific Electronic Archives. 2014;6(1): 90–96.
15. Dartora J, Marini D, Guimarães VF, Pauletti DR, Sander G. Seed Germination and seedling early growth of corn and wheat inoculated with strains of *Azospirillum brasilense* and *Herbaspirillum seropedicae*. Global Science and Technology. 2013;6(3):190-201.
16. Vogel GF, Fey R. Stimulation of germination and physiological potential of rye and Triticale by *Azospirillum brasilense*, submitted to chemical seed treatment. Scientia Agraria Paranaensis. Portuguese. 2016;15(4):493-498.
17. Sipione MS, Limede A C, Oliveira CES, Zoz A, Silva CS, Zoz T. Ways of inoculation of Azospirillum brasilense in the initial growth of triticale. Revista Scientia Agraria. 2017;18(4):86–94.
18. Bashan Y, Holquin G, De-Bashan LE. *Azospirillum*-plant relationships: physio-

logical, molecular, agricultural, and environmental advances (1997-2003). Canadian Journal of Microbiology. 2004; 8(50):521-577.

19. Piccinin GG, Braccini AL, Dan LGM, Scapim CA, Ricci TT, Bazo GL. Efficiency of seed inoculation with *Azospirillum brasilense* on agronomic characteristics and yield of wheat. Industrial Crops and Products. 2013;43:393-397.
20. Alves SRP, Francisco ALO, Carvalho TC. *Azospirillum brasilense* and nitrogen: Performance in the physiological potential of wheat seeds. Applied Research & Agrotechnology. Portuguese. 2017;10(2): 43-50.
21. Taiz L, Zeiger E, Muller IM, Murphy A. Physiology and plant development. 6th. ed. Porto Alegre: Artmed, Portuguese. 2017; 858.
22. Dartora J, Guimarães VF, Marini D, Pinto Júnior ASP, Cruz LM, Mensch R. Influence of seed treatment without initial development of maize and wheat seedlings inoculated with *Azospirillum brasilense*. Scientia Agraria Paranaensis. 2013;12(3):175-181.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23949