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SEED GERMINATION AND INITIAL DEVELOPMENT OF RICE SEEDLINGS INOCULATED WITH *Azospirillum brasilense*

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ABSTRACT - The rice crop is annual, adapted to highlands, floodplains or flooded, representing high importance for human consumption, requiring research for the optimization of cultivation and its initial development. The objective of the present work was to evaluate the germination and vigor of rice seeds, with the application of inoculant with bacteria of the species *Azospirillum brasilense*. The experimental design used was completely randomized, using six inoculant doses containing *A. brasilense*, with 4 replications of 50 seeds each, using the SCS 121 CL cultivar. Germination evaluations were carried out daily up to 14 days, the first count test on the 5th day and the second count on the 14th day after sowing. The germination speed index and the biometric parameters were also performed at the end of the experiment. The dose of 4.0 mL 1000^{-1} seeds of inoculant was the treatment that resulted in a percentage of germination in the rice seed. Root length increased with higher doses, however there was no significant difference in root dry mass. The different inoculant doses do not interfere with the development of the rice aerial part.
Keywords: *Oryza sativa*, rhizobacteria, growth promoter bacteria.

GERMINAÇÃO DE SEMENTES E DESENVOLVIMENTO INICIAL DE PLÂNTULAS DE ARROZ INOCULADAS COM Azospirillum brasilense

RESUMO - A cultura do arroz é anual, adaptada as terras altas, várzeas ou inundadas, representando elevada importância para o consumo humano, com necessidade de pesquisas para a otimização do cultivo e do seu desenvolvimento inicial. O objetivo do presente trabalho foi avaliar a germinação e o vigor de sementes de arroz, com a aplicação de inoculante com bactérias da espécie *Azospirillum brasilense*. O delineamento experimental utilizado foi inteiramente casualizado, utilizando seis doses de inoculante contendo *A. brasilense*, com 4 repetições de 50 sementes cada, utilizando a cultivar SCS 121 CL. As avaliações para germinação foram realizadas diariamente até 14 dias, o teste de primeira contagem ao 5º dia e a segunda contagem ao 14º dia após a semeadura. O índice de velocidade de germinação e os parâmetros biométricos também foram realizados ao final da experimentação. A dose de 4,0 mL 1000^{-1} sementes de inoculante foi o tratamento que resultou em uma porcentagem de germinação na semente de arroz. O comprimento da raiz aumentou com as maiores doses, no entanto não deu diferença significativa na massa seca de raiz. As diferentes doses do inoculante não interferem no desenvolvimento da parte aérea do arroz.
Palavras-chave: *Oryza sativa*, rizobactéria, bactérias promotoras de crescimento vegetal.

INTRODUCTION

Rice (*Oryza sativa* L.), belonging to the Gramineae Family (Poaceae), stands out for being the third largest crop in production in the world among cereals. In Brazil, despite its almost indispensable importance for daily meals, grain production reached 10 million tons, with a planted area of 1.6 million hectares in the 2021/22 harvest (CONAB, 2023a). However it was reduced by 7.8% in the 2022/23 harvest and one of the highlights of this reduction is the high production cost and the replacement of cultivation with soybeans and corn (CONAB, 2023b).

According to Garé et al. (2017) options have been sought that help increase efficiency in the use of inputs, reducing expenses with rice production, including biological fixation of atmospheric nitrogen (BNF). Some research reveals that rice plants are frequently colonized by microorganisms called diazotrophic bacteria, which form endophytic associations, colonizing internal spaces of the plants, contributing in terms of biological nitrogen fixation, as they can provide appropriate conditions to protect the nitrogenase complex from exposure to oxygen (SANTOS et al., 2013). In addition to FBN, these microorganisms produce growth-promoting substances in plants, capable of colonizing plant roots or the environment around them and are also called growth-promoting rhizobacteria (COSTA et al., 2015).

The substances produced by these rhizobacteria are called phytohormones, which is one of the factors, perhaps the main one, responsible for the stimulatory effect observed on plant growth, as in the

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case of inoculation with strains of *Azospirillum* sp. (COSTA et al., 2015). Over time, studies have been carried out with the aim of evaluating the beneficial aspects of the association of diazotrophic bacteria with seed germination, such as Cadore et al. (2016) observed an increase in rice seed germination using inoculation based on *Azospirillum* sp. bacteria, after 14 days of sowing. Thus, the application of rhizobacteria with the aim of promoting the germination and establishment of rice plants can be a low-cost and easy-to-adopt technology.

In view of the above, the objective of this work was to evaluate the germination and vigor of rice seeds, variety SCS 121 CL, through doses of inoculant with bacteria of the species *Azospirillum brasilense*.

MATERIAL AND METHODS

The present work was conducted at the Phytopathology Laboratory of the Pontifical Catholic University of Paraná (PUCPR), *Campus* Toledo, from September to October of 2017. The experimental design used was completely randomized, containing four replications of 50 seeds each, arranged on paper germitest and maintained in a controlled environment (BOD). The seeds used in the experiment belonged to the SCS 121 CL® variety, supplied by Empresa Sementes Vitorino®, based in Turvo/SC. The seeds were previously cleaned with a 5% sodium hypochlorite solution and dried with paper towels for subsequent inoculation. Six treatments were established for 1000 seeds with different dosages of inoculant, as follows: T1 - control, T2 - 0.8 mL, T3 - 1.6 mL, T4 - 2.4 mL, T5 - 3.2 mL and T6 4 .0 mL.

The distribution of the inoculant over the seeds occurred using a micropipette of variable volume directly in a polyethylene plastic packaging and after application, aiming at their homogenization, the seeds were stirred inside the packaging for 5 minutes together with the inoculant consisting of a solution of *Azospirillum brasilense* bacteria with strains Abv-5 and Abv-6 at concentration of 2.0 x 108 viable cells mL^{-1}.

After application of the inoculant the treatments were packaged in paper rolls using germitest paper, distributing 50 seeds on two pieces of paper and covered with a third paper. The papers were previously moistened with deionized water, 2.5 times their weight in volume and, subsequently, the four repetitions of each treatment were stored at a temperature of 25 ºC in a BOD chamber. The first count of the germination test was carried out on the 5th day after sowing (DAS) and the second on the 14th DAS. The result expressed as a percentage of normal seedlings (BRASIL, 2009). At the end of the test, the seedlings were sectioned into shoots and roots, measuring the average length of shoots and roots, expressed in cm, with the samples arranged in sequence, in a forced air oven, at 65° C, for 72 h, obtaining the weighing of the dry biomass of the aerial part and root of each seedling, expressed in g $seedling^{-1}$.

The germination speed index (GSI) was performed daily, calculated by counting the germinated seeds and, subsequently, the data was submitted to the Maguire formula (1962) (Equation 1).

$$GSI = \frac{N1}{DQ} + \frac{N2}{D2} + \frac{N3}{D3} + \cdots + Nn/|Dn|$$

(Equation 1)

Where:

N = number of seedlings verified on the day of counting;

D = number of days after sowing, in which the count was carried out.

The data obtained were subjected to analysis of variance, using the F test and when significant differences were detected, regression analysis was performed, with the aid of the Sisvar statistical program (FERREIRA, 2011).

RESULTS AND DISCUSSION

When seed germination was evaluated at 5 DAS (Figure 1A) and 14 DAS (Figure 1B), it could be observed that the percentage of germination did not differ statistically in function of treatments. Furthermore, the behavior of the germinated seeds showed better adjustment to a quadratic behavior during the period, inferring that T6 (dose of 4.0 mL of inoculant) was the treatment that resulted in the highest percentage of germination in both DAS.

Cadore et al. (2016) also found in their study that seeds of the rice cultivar IRGA 424 RI, inoculated with doses of the inoculant with Azospirillum brasilense showed higher germination rates throughout the period, thus corroborating the results of the present work. Hahn et al. (2016), also reported significant differences in the initial germination of seeds of the rice cultivar IRGA 422CL, depending on different isolates of diazotrophic bacteria associated with the Azospirillum bacteria studied. However, the same authors observed increasing linear behavior, depending on higher doses of inoculant, that is, the higher the dose of inoculant with *Azospirillum brasiliense* used the higher the germination rate. Although this behavior occurred for the two rice cultivars in both studies, there was a certain disparity between the responses to inoculation.

Different rice cultivars can express different results, depending on the same inoculum, possibly due to differences in interactions between plants and microorganisms, suggesting that each cultivar has specificity, responding unequally to the same stimulus. For Santi et al. (2013), despite the ecological and phylogenetic diversity between diazotrophic bacteria and their hosts, a well-regulated interaction between microorganisms and the host plant is essential for a successful interaction between these components to be achieved.

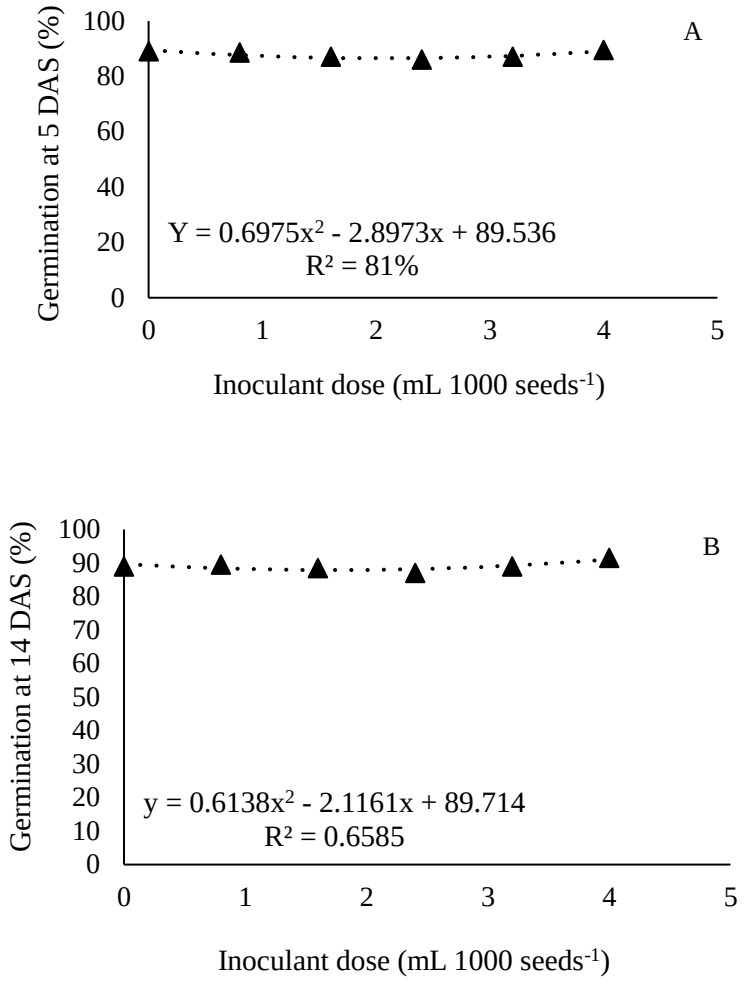

FIGURE 1 - Germination of rice seeds at 5 DAS (A), germination of rice seeds at 14 DAS (B), with application of inoculant containing *Azospirillum brasilense*.

In Figure 2A it is possible to observe that the length of the roots differed statistically between the doses of the inoculant with *A. brasilense*, obtaining a high coefficient of determination, unlike the aerial part (Figure 2B), which was not observed to be significant in its length, due to of the doses of inoculant applied, that is, the application of the inoculant had no effect on the cell growth of rice leaves.

Pankievicz et al. (2015), when studying biological nitrogen fixation in the roots of poaceae such as *Setaria viridis*, they found that, in the absence of nitrogen, some evaluated parameters did not differ, such as plant height, fresh and dry biomass of the root system. While Gholamalizadeh et al. (2017) found significant differences for the length of the aerial part and length of the roots of rice seedlings, depending on the doses of bacterial inoculums of *Alcaligenes facealis*, *Pantoea a*nanatis, *Stenotrophomonas maltophilia*, *Bacillus vietnamensis*, *Bacillus idriensis*, which are growth-promoting rhizobacteria. The authors observed an increase in seedling length and vigor index, when compared to the control treatment, concluding that all the bacteria studied had a positive influence on seedling growth, but with different potential in the treatments.

The difference found in this study, compared to other studies, may be in the susceptibility of rice depending on the bacteria used, due to its characteristic of producing indoleacetic acid (IAA) (MARTINS et al., 2020). AIA is a growth-regulating plant hormone, which acts at reduced levels in the meristematic areas of plants, thus stimulating cell development and expansion (TAIZ et al., 2017).

Pankievicz et al. (2015) also defend the fact that nitrogen-fixing rhizobacteria promote plant growth, but emphasize that this process is controversial, that is, there are doubts that biological nitrogen fixation in the associative interaction contributes to promoting seedling growth.

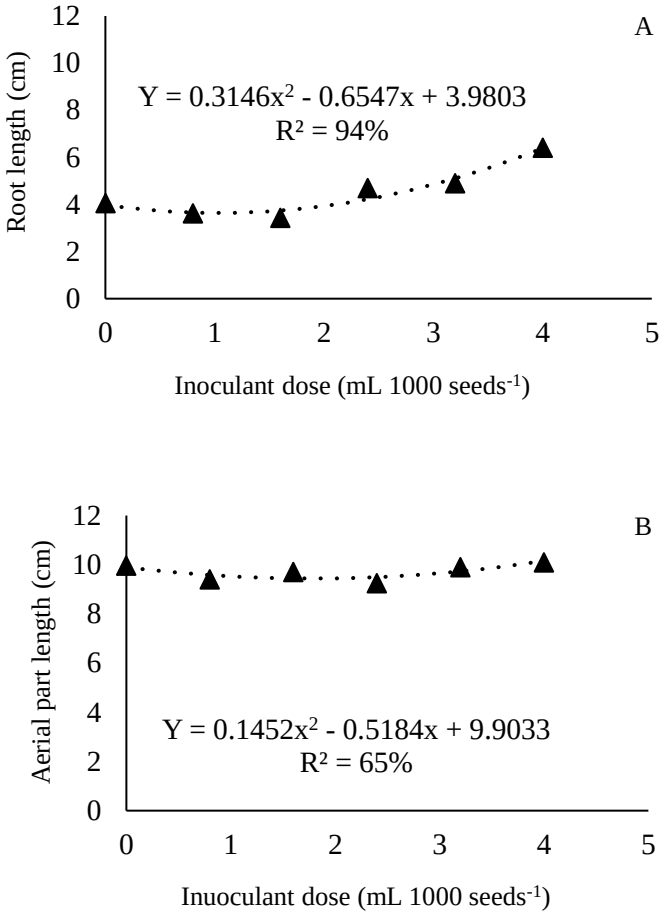

FIGURE 2 - Root length (A) and shoot length (B) of rice seedlings, as a function of doses of *Azospirillum brasilense* inoculant.

In Figure 3 it can be seen that the germination speed index (GSI) did not show significance depending on the doses of inoculant applied. The same result was also observed by Rodrigues et al. (2012), in work with inoculation of rhizobacteria in rice seedlings, which promoted an increase in the number of roots and the length of the shoot, however, without statistical difference in GSI. The GSI test aims to demonstrate how quickly treatments germinate. The higher the GSI, the stronger and more vigorous the plants will be, increasing their potential to germinate and produce healthy seedlings. This provides them with resistance to adverse environmental conditions.

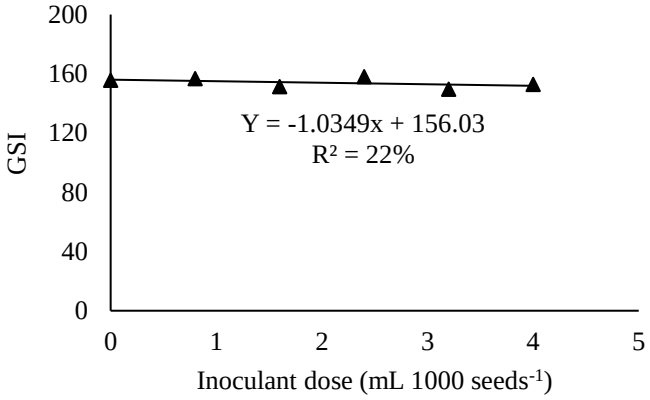

FIGURE 3 - Germination speed index (GSI) of rice seeds, with application of inoculant containing *Azospirillum brasilense.*

The dry mass of the root system and aerial part analyzed in rice seedlings did not show a statistically significant difference between the dosages of *A. brasilense*. The small difference in the dry mass of the roots (Figure 4A) and the aerial part (Figure 4B) depending on the inoculant doses may be related to the greater reserve present in the seed at the time of testing, being directed towards the development of the root system of rice seedlings. The only external addition during the experimental period was deionized water, showing no differences between treatments.

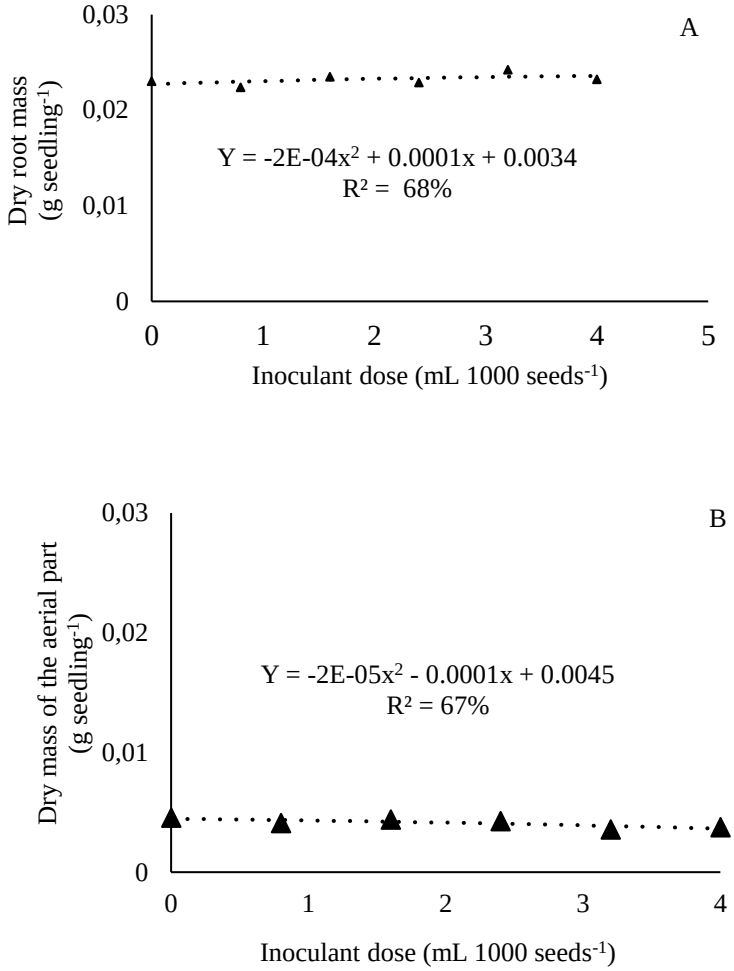

FIGURE 4 - Root dry mass (A) and shoot dry mass (B) of rice seedlings inoculated with doses of inoculant containing *Azospirillum brasilense*.

Rampim et al. (2012) evaluated the physiological quality of wheat seed varieties treated with *Azospirillum brasilense*, a fungicide and biostimulant. The bacteria provided greater dry biomass of the root system for the CD 116 variety, however, for CD 150 and CD 104 they did not show a significant difference with the control. In line with other authors, when inoculated strains of *Burkholderi*a sp. in rice cultivars such as IR42, values with an increase of up to 50% in biomass production were obtained in relation to the control, indicating that the bacterial strains have different performance in the same rice cultivar (GUIMARÃES and BALDANI, 2013).

Sabino et al. (2012), when studying the initial development of rice with diazotrophic bacteria, they found that the cultivar IAC440 showed an increase of 135.7% in fresh biomass of the root system, while the cultivar IR42 showed a corresponding decrease of 16.1%. Bianchet et al. (2015), when evaluating the vegetative development of two rice cultivars, they obtained non-significant results for the dry biomass of roots and shoots, when they added *Azospirillum* sp., with no increase in these variables. Bacterial strains may exhibit different behaviors in rice nitrogen fixation, thus making strain selection more specific.

Thus, it is clear that inoculation with growth-promoting bacteria is capable of presenting positive

effects on rice germination, however, it is necessary to carry out other studies in the laboratory, controlled environment and field, in order to investigate the consequences of these changes in physiological processes and plant development, as well as crop productivity.

CONCLUSIONS

The dose of 4.0 mL 1000^{-1} seeds of inoculant was the treatment that resulted in a percentage of germination in the rice seed.

Root length increased with higher doses, however there was no significant difference in root dry mass.

The different doses of the inoculant do not interfere with the development of the aerial part of the rice.

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