

SILICON FERTILIZATION AND SEED MICROBIOLIZATION ON DISEASE SEVERITY AND AGRONOMIC PERFORMANCE OF UPLAND RICE¹

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ABSTRACT - Rice is one of the world's most consumed cereals, however, its production is affected by fungal diseases. Thus, the objective of this work was to evaluate the severity of diseases and grain yield potential of upland rice with silicon fertilization combined with seed microbiolization with *Bacillus methylotrophicus* isolates. Two experiments were conducted, one in Igarapé do Meio, Maranhão (MA), Brazil, with rice seeds of the variety *Palha-Murcha* and one in São Bento MA with rice seeds of the variety *BRS-Primavera*. A randomized block experimental design in a split-plot arrangement with five replications were used in both experiments, with agro-silicon rates (0.0, 1.0, 2.0, 4.0 and 6.0 Mg ha⁻¹) in the plots and microbiolized and non-microbiolized rice seeds with *B. Methylotrophicus* at concentration of 10⁸ CFU ml⁻¹ in the subplots. The seedling emergence, grain yield, number of panicles, plant height, plant dry weight and severity of brown leafspot, leaf scald and grain spot were evaluated. Soil fertilization with agro-silicon affected positively the plant height of the variety *Palha-Murcha* and the number of panicles, plant dry weight and grain yield of the variety *BRS-Primavera*, and negatively the germination of the variety *BRS-Primavera*. Leaf scald severity in the variety *BRS-Primavera* reduced with microbiolized seeds with *B. methylotrophicus*. Microbiolization with *B. methylotrophicus* had no effect on severity of brown leafspot and grain spot in the varieties evaluated.

Keywords: *Bacillus methylotrophicus*. Agrosilicon. Biological control. Diseases. Rice production.

EFEITO DA FERTILIZAÇÃO DE SILÍCIO E MICROBIOLIZAÇÃO DE SEMENTES SOBRE A SEVERIDADE DE DOENÇAS E DESEMPENHO AGRONÔMICO DE ARROZ DE SEQUEIRO

RESUMO - O arroz é um dos cereais mais consumidos em todo o mundo e sua produção é afetada por doenças fúngicas. Neste sentido objetivou-se avaliar a severidade de doenças e o potencial produtivo do arroz de terras altas em função da aplicação de silício e sementes microbiolizadas com isolados de *Bacillus methylotrophicus*. Foram realizados dois experimentos, em dois municípios do estado do Maranhão, Brasil: um em Igarapé do Meio, com sementes de arroz da variedade Palha Murcha e outro em São Bento, com a variedade Primavera. Os experimentos foram instalados no delineamento em blocos casualizados, com cinco repetições, em parcelas subdivididas. As parcelas foram constituídas por cinco doses de agrosilício (0,0; 1,0; 2,0; 4,0 e 6,0 t ha⁻¹) e as subparcelas foram constituídas por sementes de arroz microbiolizadas e não microbiolizadas com *B. methylotrophicus*, na concentração de 10⁸ UFC ml⁻¹. Os parâmetros avaliados foram emergência de plântulas, produtividade de grãos, número de panícula, altura de planta, massa de planta seca e severidade de mancha parda, escaldadura e mancha de grãos. As doses de agrosilício aplicadas no solo afetaram positivamente a altura de plantas, para a variedade Palha Murcha. Na variedade Primavera, as doses de agrosilício influenciaram de forma positiva o número de panículas, massa de planta seca e a produtividade de grãos e de forma negativa a germinação. Para esta variedade, a severidade da escaldadura foi reduzida quando as sementes foram microbiolizadas com *B. methylotrophicus*. A microbiolização com *B. methylotrophicus* não influenciou a severidade da mancha parda e a severidade da mancha de grãos, para as duas variedades de arroz.

Palavras-chave: *Bacillus methylotrophicus*. Agrosilício. Controle biológico. Doenças. Produção de arroz.

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¹Received for publication in 08/25/2016; accepted in 04/10/2017.

Paper extracted from the doctoral thesis of the first author.

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INTRODUCTION

Genetically improved rice varieties have grain yield potential of 10 Mg ha⁻¹, however, their world average grain yield is 5 Mg ha⁻¹, which denotes the need for reduce this difference in grain yield observed in the field. This difference is due mainly to biotic and abiotic stresses that affect rice crops (KHUSH; JENA, 2008). Fungal diseases are among this biotic factors, affecting the rice grain quality and yield.

Various fungal diseases affect upland rice, such as the leaf blast (*Pyricularia oryzae* Cav.), leaf scald (*Monographella albescens* Thume), brown leafspot (*Helminthosporium oryzae* Breda de Haan), grain spot (*Phoma* spp., *Drechslera oryzae* Breda de Haan, *Curvularia lunata* (Wakker) Boedijn, *Nigrospora oryzae* (Berk. & Broome), *Alternaria* spp. and *Fusarium* spp.) and narrow brown leafspot (*Cercospora oryzae* Miyake) (UTUMI, 2008). Leaf scald symptoms begin in the apical parts or blade edges of the leaves, with olive-green coloration. Brown leafspot symptoms occur in leaves, grains, coleoptile, panicle branches and sheath, showing reddish-brown oval spots, with gray center. Grain spot symptoms are variable, depending on the pathogen, infection stage and climatic conditions. These diseases are affected by environmental conditions, plant density, dew (SANTOS; SANTIAGO, 2014) and plant nutritional imbalance (SANTOS et al., 2010). The diseases leaf scald, brown leafspot and grain spot, evaluated in the present work, occur in crops in the State of Maranhão, Brazil.

Silicon fertilization as phytosanitary control of diseases are of interest to researchers and society, since it reduces applications of fungicides, improves growth, development and architecture and strengthens cell wall of plants (GOMES et al., 2011). Moreover, it reduces cuticular transpiration, increases photosynthetic efficiency, resistance to pest and pathogens and tolerance to drought of plants and promotes formation of compounds such as phenols (MENDONÇA et al., 2013). It also potentiates activities of enzymes such as peroxidases, polyphenoloxidases and quinonases (GOMES et al., 2008), increases the action of anti-oxidative defenses, reduces oxidative damage of functional molecules in membranes and increases stomatal resistance (GUNES et al., 2007; CRUSCIOL et al., 2009).

Silicon fertilization may affect rice grain yield, control or reduce diseases such as brown leafspot (SANTOS et al., 2011) and reduce leaf blast severity in leaves and panicles (FILIPPI et al., 2012). The reduction of disease severity is due to the absorbed silicon deposited below the cuticle, which forms a double layer silica-cuticle (SANGSTER; HODSON; TUBB, 2001).

Bacillus methylotrophicus is a gram-positive

bacterium that interacts well with plants, has high guanine and low cytokinins contents, excellent effect for disease biological control, plant growth promotion and bioremediation, and produces bacteriocins, which makes its use as a plant protection agent a potential alternative for disease control (TUMBARSKI; PETKOV; DENKOVA, 2015).

Rice seeds microbiolization with *Pseudomonas fluorescens* (Flugge) Migula, *Bacillus subtilis* (Cohn), *Bacillus* spp. and *Stenotrophomonas malthophilia* (Swings) is considered an efficient treatment to control *Rhizoctonia solani* Kuhn, due to the ability of these fungi to control this pathogen and the possibility of make them more efficient by combining them with compounds to stimulate their activity (LUDWIG; MOURA, 2007).

The hypothesis considered in this work is that silicon fertilization combined with the seed microbiolization with *B. methylotrophicus* reduces the difference between the grain yield potential and observed in the field, by reducing the plant susceptibility to pathogens in rice crops.

Thus, the objective of this work was to evaluate the severity of diseases and grain yield potential of upland rice with silicon fertilization combined with seed microbiolization with *B. methylotrophicus* isolates.

MATERIAL AND METHODS

Two experiments were conducted in rice fields with history of leaf blast, leaf scald, brown leafspot and grain spot; one in the rural settlement Diamante Negro, Jutai, Igarapé do Meio, State of Maranhão, Brazil (03°35'9"N, 45°09'50.6"W and 9 m of altitude) in the 2013-2014 crop season, and one in the School Farm of the Maranhão State University, São Bento, State of Maranhão, Brazil (2°42'15,6"N, 44°51'19,6"W and 2 m of altitude) in the 2014-2015 crop season.

The rice variety *Palha-Murcha* (Creole) was planted in Igarapé do Meio and the variety *BRS-Primavera* (improved) in São Bento. The variety *BRS-Primavera* is indicated for areas of low to moderate fertility, due to its tendency to lodging in areas of high fertility, reaching grain yields of up to 3,550 kg ha⁻¹, and is considered moderately resistant to the diseases brown leafspot and grain spot (EMBRAPA, 2014). The average temperature of the two experimental areas was 31.7 °C and their mean precipitation was 218 mm (Meteorology Laboratory of the Geo-Environmental Center of the Maranhão State University).

The soils of the experimental areas were collected to evaluate their silicon content and determine their chemical and physical characteristics. The soils of the experimental areas Igarapé do Meio and São Bento had respectively

sandy loam and loamy sand texture, 27 and 10 g dm⁻³ of organic matter, pH based on CaCl₂ of 4.3 and 4.3, 4.3 and 4.0 mg dm⁻³ of P, 3.7 and 3.5 mmol_c dm⁻³ of K⁺, 37 and 12 mmol_c dm⁻³ of Ca²⁺, 22 and 10 mmol_c dm⁻³ of Mg²⁺, 42 and 23 mmol_c dm⁻³ of H⁺+Al³⁺, 69.4 and 25.5 mmol_c dm⁻³ of sum of bases, 111.4 and 48.5 mmol_c dm⁻³ of cation exchange capacity, 62.5% and 53% of base saturation and 5.9 and 1.5 mg dm⁻³ of silicon.

Silicon fertilization with agro-silicon (25% Ca, 6% Mg and 10.5% Si) and liming with dolomitic limestone (32% CaO, 15% MgO, total neutralizing power of 90%) were performed 60 days before the rice planting. The agro-silicon applied (6.0 Mg ha⁻¹) contained 1,500 kg ha⁻¹ of Ca and 360 kg ha⁻¹ of Mg, according to its Ca, Mg and Si contents. Dolomitic limestone was applied to balance Ca and Mg contents of the experimental units, thus isolating the effect of the silicon released by the agro-silicon, since both the limestone and agro-silicon are used as sources of Ca and Mg.

A randomized block experimental design in a split-plot arrangement with five replications were used in both experiments, with agro-silicon rates (0.0, 1.0, 2.0, 4.0 and 6.0 Mg ha⁻¹) applied 60 days before planting in the plots and microbiolized and non-microbiolized rice seeds with *Bacillus Methylophilicus* (isolates B12 and B41) in the subplots.

The plots had area of 6.0 m x 4.5 m and the subplot had area of 6.0 m x 2.25 m. The two rice varieties were grown in the upland rice system, with spacing of 0.45 m between rows, and 360,000 plants ha⁻¹. The evaluation area of the subplots was 2.7 m², discarding the two lateral rows and the borders (2.0 m of each end of the central rows).

The isolates of *B. methylophilicus* (B12 and B41) used for the rice seed microbiolization with molecular identification are part of the biological collection of the Phytopathology Laboratory of the Maranhão State University, registered as MGSS-214 and MGSS-215, respectively, which have proved antimicrobial activity in other pathological systems. These isolates were evaluated in vitro and inhibited mycelial growth of *Magnaporthe oryzae* (NASCIMENTO et al., 2016).

These *B. methylophilicus* isolates were multiplied in culture medium (PDA) and maintained in a BOD chamber for growth for 48 hours at 28 °C and 12-hour photoperiod. Then, bacterial suspensions were prepared with these isolates, adjusting their concentrations to 10⁸ UFC ml⁻¹ in a spectrophotometer (wavelength of 540 nm and absorbance of 0.5). The two isolates were mixed, using 1000 mL of the B12 and B41 inocula. Seeds of the two rice varieties were immersed for 24 hours under constant stirring at 50 rpm in this mix.

Manual and mechanical weeding were

performed in the experimental area of Igarapé do Meio before planting, and manual weeding were performed throughout the experimental periods.

Soil fertilization at planting consisted of 200 kg ha⁻¹ of 5-30-10 (N-P-K) (Igarapé do Meio) and 200 kg ha⁻¹ of 5-30-15 (N-P-K) (São Bento), based on the soil chemical analysis and commercial formulations locally available. Rice plants of the variety *BRS-Primavera* (São Bento) were fertilized with 91 kg ha⁻¹ of urea (45% of N) 40 days after emergence due to nitrogen deficiency symptoms in the plants. Rice plants of the variety *Palha-Murcha* (Igarapé do Meio) showed no symptoms of nitrogen deficiency.

The number of emergent seedlings was manually counted at the 5th, 10th and 15th day after planting. Ten plants per subplot of the variety *BRS-Primavera* were collected in these same three days, to measure their height (mm) from the sheath to the insertion of the last fully expanded leaf. Plant height (variety *Palha-Murcha*) and dry weight (both varieties) were evaluated after 130 (*Palha-Murcha*) and 110 (*BRS-Primavera*) days of planting, using 10 plants per subplot. The collected plants were packed in paper bags, dried in a forced air circulation oven at 70 °C and weighed to obtain the plant dry weight (kg ha⁻¹). The number of panicles in the evaluation area of the subplot (2.7 m²) was counted (units m⁻²) at the end of the crop cycle and its grains were removed manually, stored in paper bags and weighed in a precision scale (kg ha⁻¹) with water content corrected to 13%.

Leaf scald and brown leafspot severity were evaluated in Igarapé do Meio at the last stage of the plant cycle (110 days after emergence); these diseases have high occurrence at this stage. Leaf scald was evaluated in São Bento at the floral differentiation stage and brown leafspot at the last stage of the plant cycle (103 days after planting).

Ten leaves were randomly collected in each subplot to evaluate these two diseases, using the scale of grades of the Centro Internacional de Agricultura Tropical – CIAT (1983) considering the leaf area affected by the diseases (0 = absence of diseases, 1 = below 1%, 3 = 1 to 5%, 5 = 6 to 25%, 7 = 26 to 50%, 9 = above 50%). Leaf scald severity was evaluated considering the lesion length in relation to the total area in each leaf. Grain spot severity was evaluated on 400 grains removed from eight panicles collected from each subplot, using a scale of grades considering the stained surface of the grain (0 = none, 1 = 1 to 25%, 2 = 26 to 50%, 4 = 51 to 75%) (SILVA-LOBO et al., 2011).

The rice varieties were analyzed separately. Data were subjected to analysis of variance and regression. The models were chosen based on the biological phenomenon, significance of the regression coefficients (using probability levels of up to 10%), t-test and coefficient of determination ($R^2 = \text{SQ}_{\text{Regression}} / \text{SQ}_{\text{Treatment}}$). The means of all the

variables of the microbiolized and non-microbiolized seeds were compared by the F test at 5% of probability level. The Statistical Analysis were performed using the program SAEG 9.1 (SAEG, 2007).

RESULTS AND DISCUSSION

The interaction between seed microbiolization and agro-silicon rates had no significant effect on the seedling emergence (SE, $p=0.6426$ and $p=0.2829$), grain yield (GY, Kg ha^{-1} , $p=0.4390$ and $p=0.4150$), plant height (PH, $p=0.1161$ and $p=0.1160$), plant dry weight (PDW, $p=0.1062$ and $p=0.9999$), severity of brown leafspot (BL, $p=0.8469$ and $p=0.9999$) leaf scald (LS, $p=0.9508$ and $p=0.2218$) and grains spot (GS, $p=0.3595$ and $p=0.9999$) in the varieties *Palha-Murcha* and *BRS-Primavera*, and on the number of panicle (NP, $p=0.8120$) of the variety *Palha-Murcha*. Therefore, the effect of agro-silicon and seed microbiolization was assessed separately.

The interaction between seed microbiolization and agro-silicon rates had significant effect on the NP ($p=0.0316$) of the variety *BRS-Primavera*, increasing linearly with increasing agro-silicon rates with microbiolized seeds (Figure 1A) and showed a quadratic effect with non-microbiolized seeds (Figure 1B). The NP ($F_{1,20}=6.49$; $p=0.0192$) was affected by the seed microbiolization with *Bacillus methylotrophicus*. The highest NP was found in plants of non-microbiolized seeds (Table 1). This result may be related to permanence for 24 hours of the microbiolized seeds in the *B. methylotrophicus* solution, which reduced the emergence of seedlings. Similar results were found by Filippi et al. (2012), with all silicon rates reducing leaf blast severity in leaves (area under the disease progress curve - AUDPC) and panicles, and increasing the weight of one thousand grains. These authors also found a reduction of 76 and 50% of AACPD, respectively with and without microbiolized seeds, denoting the positive interaction between silicon fertilization and rhizobacteria.

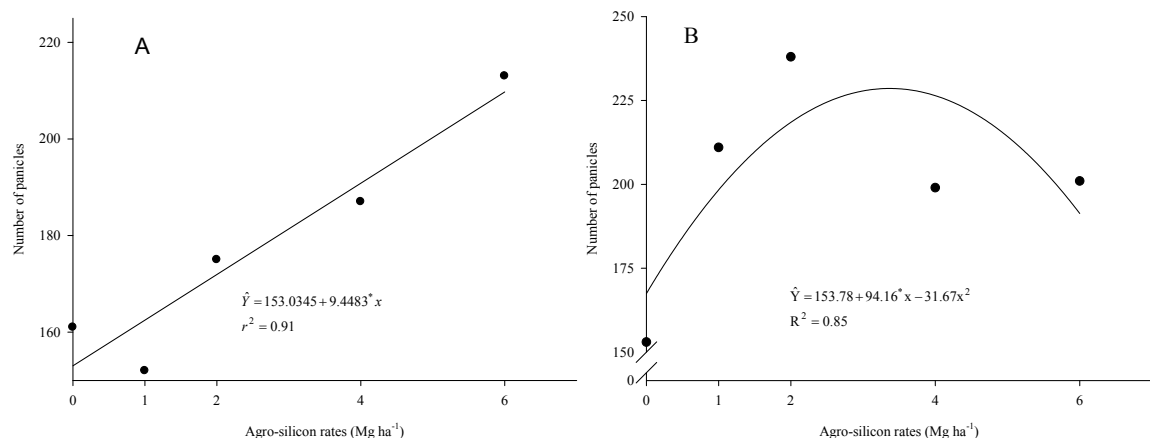


Figure 1. Number of panicles as a function of agro-silicon rates (Mg ha^{-1}) on microbiolized (A) and non-microbiolized (B) seeds of the rice variety *BRS-Primavera* with *Bacillus methylotrophicus*, in the 2014-2015 crop season.

The height of plants (PH) of the variety *Palha-Murcha* increased linearly with increasing agro-silicon rates (Figure 2), increasing 1.99 cm for each Mg of agro-silicon applied. The PH of the variety *BRS-Primavera* was not affected by the agro-silicon rates, showing mean PH of 18.12 cm. The results of the improved variety (*BRS-Primavera*) confirm those found by Reis et al. (2008), who evaluated silicon fertilization in two rice varieties (IAC 201 and IAC 202) with aspersion irrigation and found no effect on plant height.

The agro-silicon rates had no effect on the SE, GY, PDW and NP of the variety *Palha-Murcha*, whose averages were 19.99% (SE), 567.51 kg ha^{-1} (GY), 3348 kg ha^{-1} (PDW) and 22.4 (NP). The SE (decreasing linear effect), PDW (increasing linear

effect) (Figure 3A) and GY (plateau effect) of variety *BRS-Primavera* were affected with increasing agro-silicon rates (Figure 3B). The model for SE as a function of agro-silicon rates was $\hat{Y} = 21.04 - 0.4023 \cdot x$; $r^2 = 0.70$. The GY reached the plateau at 1,514.2 kg ha^{-1} , with a agro-silicon rate of 2.56 Mg ha^{-1} . According to the decreasing increments law, fertilizers should not be applied aiming the maximum yield, but a yield that returns the greatest profit for the farmer, i.e., the maximum economic yield. Therefore, following this principle of the law of decreasing increments when increasing a high potassium silicate rates, productivity has a tendency to not follow this increase, since the maximum economic productivity was reached (SANTOS et al., 2010).

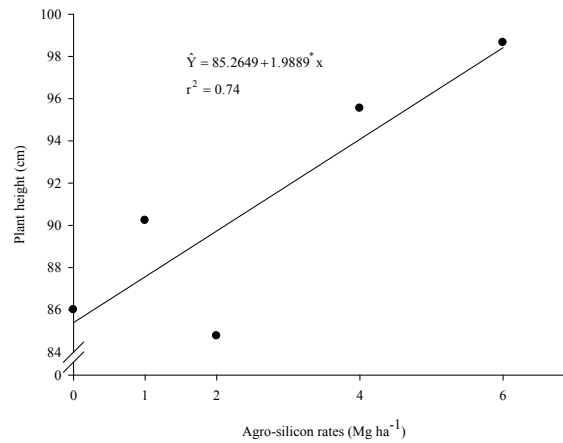


Figure 2. Plant height (cm) of the rice variety *Palha-Murcha* as a function of agro-silicon rates (Mg ha⁻¹) in the 2013-2014 crop season.

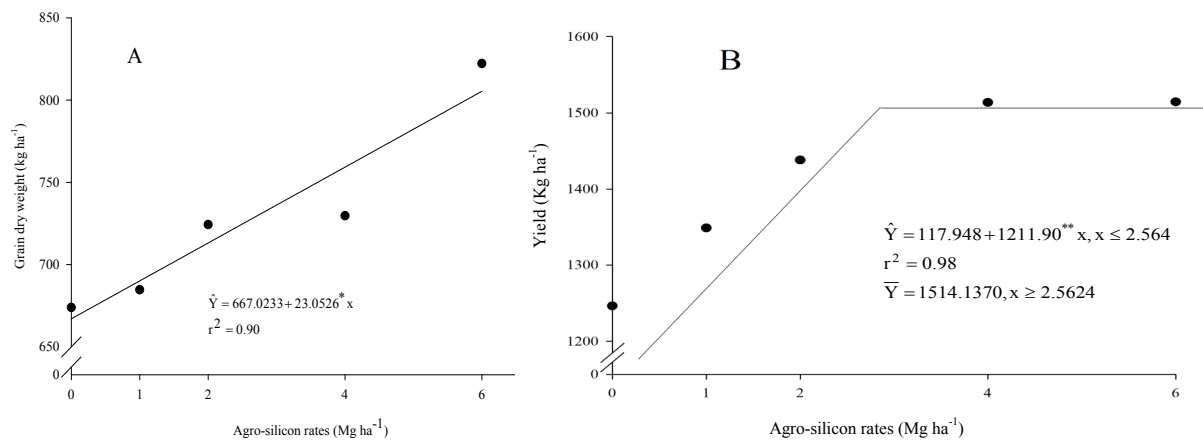


Figure 3. Grain dry weight (A) and yield (B) of the rice variety *BRS-Primavera* as a function of agro-silicon rates (Mg ha⁻¹) in the 2014-2015 crop season.

The grain spot severity (*Phoma* sp., *Drechslera oryzae*, *Curvularia lunata*, *Nigrospora oryzae*, *Alternaria* sp., *Fusarium* sp.) had a decreasing quadratic effect on the variety *Palha-Murcha* (Figure 4A) and decreasing linear effect (Figure 4B) on the variety *BRS-Primavera*

with increasing agro-silicon rates. The agro-silicon rates had no effect on the brown leafspot and leaf scald severities, which showed means of 1.17 and 1.84 (*Palha-Murcha*) and 1.48 and 1.87 (*BRS-Primavera*), respectively.

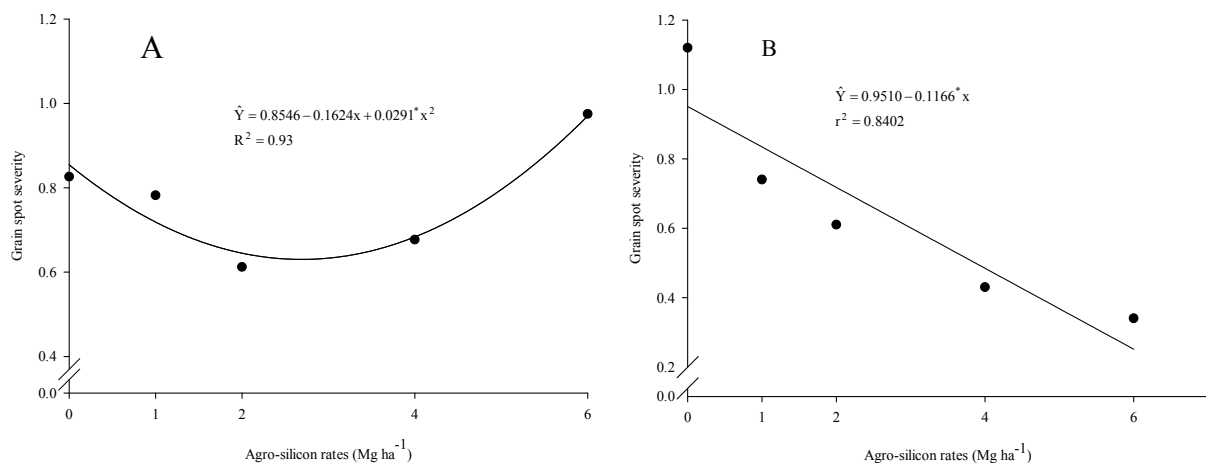


Figure 4. Grain spot severity in the rice variety *Palha-Murcha* (Igarapé do Meio, State of Maranhão, Brazil) and *BRS-Primavera* (B) as a function of agro-silicon rates (Mg ha⁻¹) in the 2013-2014 (*Palha-Murcha*) and 2014-2015 (*BRS-Primavera*) crop season.

The silicon content in the soil probably affected the agronomic performance and severity of diseases of the upland rice varieties. The soil of Igarapé do Meio (*Palha-Murcha*) had 5.9 mg dm⁻³ of silicon, which may have hindered the response of the plants; and the soil of São Bento (*BRS-Primavera*) had 1.5 mg dm⁻³ of silicon, favoring the response of the cultivar to silicon fertilization. Marchezan et al. (2004) found no response of lowland rice to silicon fertilization using calcium silicate in a soil containing 5 to 12 mg L⁻¹ of silicon. On the other hand, Chagas et al. (2016) found an increase in rice grain yield with application of silicon. Mauad et al. (2013) found different plant growth for two rice cultivars fertilized with calcium silicate and dolomitic limestone; the cultivar Maravilha had higher shoot dry matter production in the anthesis and milky grain stages and the cultivar Caiapó had higher shoot dry matter production in the physiological maturation stage.

The amount of calcium applied was adequate with agro-silicon rates of 1.0, 2.0, 4.0 and 6.0 Mg ha⁻¹ combined with limestone, and sufficient to increase the rice yield of the variety *Palha-Murcha*. Silva and Bohnen (2003) found similar results, with no beneficial effects of silicon with adequate rates of Ca on the evaluated parameters. Thus, beneficial effects of silicon fertilization on upland rice should not be attributed to silicon alone, but to its wide action as corrective of acidity, which promotes better soil chemical conditions to plant development (BARBOSA FILHO; PRABHU, 2002).

The soil sandy texture was also an important factor in both locations. The sand fraction is constituted mainly by SiO₂ quartz, which has low release of silicon to plants and greater drainage, favoring silicon losses by leaching. According to

Marchezan et al. (2004), soil chemical and physical composition and flood conditions affect silicon availability and plant responses. This information is confirmed by Camargo, Kondorfer and Côrrea (2002), who reported more availability of silicon to plants with increasing soil clay content.

On the other hand, the variety *Palha-Murcha* is not improved and it may not respond to silicon treatments due to its genetic characteristics, while the variety *BRS-Primavera* is from a modern group, has small plants, is indicated for planting in soils of little to moderate fertility, and is moderately resistant to brown leafspot and grain spot (EMBRAPA, 2014). The ability to absorb nutrients from the soil and nutrient demand required vary according to several factors, including the cultivar (FARIA JÚNIOR et al., 2009).

The microbiolization had no effect on the GY [(F_{1,20}=0.025; p=0.8766) and (F_{1,20}=0.78; p=0.9999)], PH [(F_{1,20}=2.372; p=0.1161) and (F_{1,20}=2.141; p=0.1586)] (Table 1), BL severity [(F_{1,20}=1.696; p=0.2076) and (F_{1,20}=0.781; p=0.9999)] and GS severity [(F_{1,20}=1.614; p=0.2185) and (F_{1,20}=1.456; p=0.2417)] of the *Palha-Murcha* and *BRS-Primavera*, respectively (Table 2).

Seed microbiolization had no effect on the SE (F_{1,20}=2.135; p=0.1595), PDW (F_{1,20}=0.327; p=0.5735), NP (F_{1,20}=1.880; p=0.1855) (Table 1) and LS (F_{1,20}=1.835; p=0.1907) of the variety *Palha-Murcha* (Table 2). These same variables were affected by the seed microbiolization with *B. methylotrophicus*, in the variety *BRS-Primavera*, with SE (F_{1,20}=33.87; p<0.0001), PDW (F_{1,20}=5.42; p=0.0305) (Table 1) and LS (F_{1,20}=4.66; p=0.0433) (Table 2). The rice seed microbiolization with *B. methylotrophicus* reduced the leaf scald severity in the variety *BRS-Primavera* by 40% (Table 2).

Table 1. Mean values of agronomic variables of the rice varieties *Palha-Murcha* and *BRS-Primavera* as a function of microbiolized and non-microbiolized seeds with *B. methylotrophicus*.

Microbiolization	<i>Palha-Murcha</i>				
	SE (%)	GY (kg ha ⁻¹)	NP (unit m ⁻²)	PH (cm)	PDW (kg ha ⁻¹)
With	31.9 a	564.5 a	57.6 a	92.1 a	3.452.4 a
Without	35.7 a	570.4 a	63.6 a	88.7 a	3.243.6 a
CV _{plot} (%)	18.2	60.1	47.1	15.3	55.6
CV _{subplot} (%)	27.2	23.3	25.2	M 8.5	39.1
<i>BRS-Primavera</i>					
With	43.8 b	1356.0 a	178.0 b	21.00 a	759.6 a
Without	56.6 a	1698.5 a	200.0 a	17.00 a	684.0 b
CV _{plot} (%)	24.1	39.8	45.7	16.6	22.5
CV _{subplot} (%)	16.2	24.5	16.7	21.3	17.2

Means followed by same letter in the columns do not differ by the F test at 5% probability. SE = seedling emergence, GY = grain yield, NP = number of panicles, PH = plant height, PDW = plant dry weight.

Table 2. Severity of the diseases leaf scald (LS) (*Monographella albescens* Thume), brown leafspot (BL) (*Helminthosporium oryzae* Breda de Haan) and grain spot (GS) (*Phoma* sp., *Drechslera oryzae*, *Curvularia lunata*, *Nigrospora oryzae*, *Alternaria* sp., *Fusarium* sp.) in the rice varieties *Palha-Murcha* and *BRS-Primavera* as a function of microbiolized and non-microbiolized seeds with *B. methylotrophicus*.

Microbiolization	<i>Palha-Murcha</i>			<i>BRS-Primavera</i>		
	LS	BL	GS	LS	BL	GS
With	1.3 a	1.1 a	0.74 a	1.7 b	1.5 a	1.3 a
Without	1.4 a	1.0 a	0.79 a	2.1 a	1.5 a	1.3 a
CV _{plot} (%)	41.7	47.9	37.9	96.2	70.1	47.0
CV _{subplot} (%)	41.8	30.6	20.1	84.9	22.2	36.7

Means followed by same letter in the columns do not differ by the F test at 5% probability.

According to Ludwig, Moura and Gomes (2013), *Bacillus* spp. did not improve the growth of rice plants, however, Souza Júnior et al. (2010) reported that a mixture of *Bacillus* spp. and *Pseudomonas synxantha* increased rice shoot dry weight and root fresh weight.

Bacillus subtilis as strains related to *Bacillus amyloliquefaciens* and *Bacillus methylotrophicus* can improve plant growth by increasing hormone levels, favoring the availability of phosphate in the soil (PANE et al., 2012). The characteristics of the varieties *Palha-Murcha* and *BRS-Primavera* were different when treated with *B. methylotrophicus*. Similar result was found by Ferreira, Knupp and Martin-Didonet (2014), who inoculated six rice varieties with *Pseudomonas*, *Burkholderia* and *Rhizobium* and found significant differences in plant height and shoot fresh weight. According to these authors, rice cultivars respond differently to inoculation with growth-promoting bacteria.

The results found in the present work are similar to those found by Ludwig et al. (2009), who found a decrease in leaf scald of 63.2% with the isolate DFs416 (*Bacillus* sp.). Rhizobacteria inoculation, directly or indirectly, favors the reduction of using chemical pesticides, promoting economic benefits and also ecological benefits, since such products often generate problems of environmental contamination. According to Corrêa et al. (2008), the response to biocontrol varies depending on environmental conditions, crop and pathogen population diversity, which may have several mechanisms, such as antibiosis and production of enzymes, siderophores and antibiotics, which potentiate them as bio-controllers (GERBORE et al., 2016; PANE et al., 2012). Therefore, the results found in the present work for the varieties of *Palha-Murcha* and *BRS-Primavera* confirm the instability of biocontrol under different climatic conditions, soil microbiota, cultivar and management.

Little researches are found on soil silicon fertilization combined with seed protection with microorganisms, evaluating productive performance and severity of diseases, thus, the present work contributes with new results, since the varieties showed different responses to silicon fertilization

and seed microbiolization with *Bacillus methylotrophicus*, regarding their agronomic performance and severity of diseases.

CONCLUSION

The agro-silicon rates used affected positively the plant height of the variety *Palha-Murcha*, increased the seedling emergence, number of panicles and grain dry weight and yield of the variety *BRS-Primavera*, and reduce the grain spot severity in the varieties *Palha-Murcha* and *BRS-Primavera*.

The seed microbiolization with *Bacillus methylotrophicus* reduced the leaf scald severity in the variety *BRS-Primavera* and did not affect the brown leafspot and grain spot severity in the two rice varieties.

ACKNOWLEDGEMENTS

The authors thank the Foundation for Research and Scientific and Technological Development of Maranhão for financial support.

REFERENCES

- BARBOSA FILHO, M. P.; PRABHU, A. S. **Aplicação de silicato de cálcio na cultura do arroz**. Santo Antônio do Goiás: Embrapa Arroz e Feijão, 2002, 4 p. (Documento Embrapa Arroz e Feijão, 51).
- CAMARGO, M. S.; KORNDORFER, G. H.; CORRÊA, G. F. Características físicas e disponibilidade de silício em solos sob vegetação de Cerrado. In: REUNIÃO BRASILEIRA DE FERTILIDADE DO SOLO E NUTRIÇÃO DE PLANTAS, 25, REUNIÃO BRASILEIRA SOBRE MICORRIZAS, 9, SIMPÓSIO BRASILEIRO DE MICROBIOLOGIA DO SOLO, 7, REUNIÃO BRASILEIRA DE BIOLOGIA DO SOLO, 4, 2002, Rio de Janeiro. **Anais...** Rio de Janeiro: Fertibio, 2002.

- CENTRO INTERNACIONAL DE AGRICULTURA TROPICAL – CIAT. **Sistema de evaluación estandar para arroz**. 2. ed. Cali, 1983. 61 p.
- CHAGAS, R. C. S. et al. Silicon fertilization improve yield and quality of rice and pearl millet in cerrado soils. **Bioscience Journal**, Uberlândia, v. 32, n. 4, p. 899-907, 2016.
- CORRÊA, B. O. et al. Influência da microbiolização de sementes de feijão sobre a transmissão de *Colletotrichum lindemuthianum* (Saac e Magn.). **Revista Brasileira de Sementes**, Lavras, v. 30, n. 2, p. 156-163, 2008.
- CRUSCIOL, C. A. C. et al. Effects of silicon and drought stress on tuber yield and leaf biochemical characteristics in potato. **Crop Science**, Madison, v. 49, n. 3, p. 949-954, 2009.
- EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA. **Catálogo de cultivares de arroz**. Santo Antônio de Góias, GO: Embrapa Arroz e Feijão, 2014. 11 p.
- FARIA JÚNIOR, L. A. et al. Produção de matéria seca, teor e acúmulo de silício em cultivares de arroz sob doses de silício. **Ciência e Agrotecnologia**, Lavras, v. 33, n. 4, p. 1034-1040, 2009.
- FERREIRA, E. P. B.; KNUPP, A. M.; MARTIN-DIDONET, C. C. G. Crescimento de cultivares de arroz (*Oryza sativa* L.) influenciado pela inoculação com bactérias promotoras de crescimento de plantas. **Bioscience Journal**, Uberlândia, v. 30, n. 3, p. 655-665, 2014.
- FILIPPI, M. C. C. et al. Indução de resistência e promoção de crescimento em arroz por agentes biológicos. In: VI REUNIÃO BRASILEIRA SOBRE INDUÇÃO DE RESISTÊNCIA EM PLANTAS A PATÓGENOS, 2012, Viçosa. **Anais...** Viçosa: Suprema Gráfica e Editora Ltda, 2012. p. 51-78.
- GERBORE, J. et al. Complete Genome Sequence of *Bacillus methylotrophicus* Strain B25, a Potential Plant Growth-Promoting Rhizobacterium. **Genome Announcements**, Washington, v. 4, n. 2, p. 1-2, 2016.
- GOMES, F. B. et al. Uso do silício como indutor de resistência em batata a *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). **Neotropical Entomology**, Botucatu, v. 37, n. 2, p. 185-190, 2008.
- GOMES, C. F. et al. Disponibilidade de silício para a cultura do arroz, em função de fontes, tempo de incubação e classe de solo. **Pesquisa Agropecuária Tropical**, Goiânia, v. 41, n. 4, p. 531-548, 2011.
- GUNES, A. et al. Influence of silicon on antioxidant mechanisms and lipid peroxidation in chickpea (*Cicer arietinum* L.) cultivars under drought stress. **Journal of Plant Interactions**, Londres, v. 2, n. 2, p. 105-113, 2007.
- KHUSH, G. S.; JENA, K. K. Current status and future prospects for research on blast resistance in rice (*Oryza sativa* L.). In: WANG, G. L.; VALENT, B. (Eds.). **Advances in genetics, genomics and control of rice blast disease**. China, 2008, 428 p.
- LUDWIG, J.; MOURA, A. B. Controle biológico da queima-das-bainhas em arroz pela microbiolização de sementes com bactérias antagonistas. **Fitopatologia Brasileira**, Brasília, v. 32, n. 5, p. 381-386, 2007.
- LUDWIG, J.; MOURA, A. B.; GOMES, C. B. (Potencial da Microbiolização de sementes de arroz com rizobactérias para o biocontrole do nematoide das galhas. **Tropical Plant Pathology**, Brasília, v. 38, n. 3, p. 264-268, 2013.
- LUDWIG, J. et al. Microbiolização de sementes para o controle da mancha parda e da escaldadura em arroz irrigado. **Tropical Plant Pathology**, Brasília, v. 34, n. 5, p. 322-328, 2009.
- MARCHEZAN, F. et al. Aplicação de silício em arroz irrigado: efeito nos componentes de produção. **Bioscience Journal**, Uberlândia, v. 20, n. 3, p. 125-131, 2004.
- MAUAD, M. et al. Acúmulo de silício na parte aérea de cultivares de arroz de terras altas afetado pela aplicação de silicato e carbonato no solo. **Semina: Ciências Agrárias**, Londrina, v. 34, n. 5, p. 2049-2060, 2013.
- MENDONÇA, A. O. et al. Acúmulo de silício e compostos fenólicos na parte aérea de plantas de trigo após a adubação silicatada. **Bioscience Journal**, Uberlândia, v. 29, n. 5, p. 1154-1162, 2013.
- NASCIMENTO, I. O. et al. Isolation, identification and in vitro evaluation of *Bacillus* spp. In control of *Magnaporthe oryzae* comparing evaluation methods. **African Journal of Agricultural Research**, Lagos, v. 11, n. 19, p. 1743-1749, 2016.
- REIS, M. A. et al. Aplicação de silício em arroz de terras altas irrigado por aspersão. **Acta Science Agronomica**, Maringá, v. 30, n. 1, p. 37-43, 2008.
- PANE, C. et al. Novel strain of *Bacillus* isolated from compost and compost-amended soils, as biological control agents against soil-borne

phytopathogenic fungi. **Biocontrol Science Technology**, Londres, v. 22, n. 12, p. 1373-1388, 2012.

SAEG. **Sistema para análises estatísticas**, versão 9.1. Viçosa: UFV, 2007.

SANGSTER, A. G.; HODSON, M. J.; TUBB, H. J. Silicon deposition in higher plants. In: DATNOFF, L. E.; SNYDER, G. H.; KORNDORFER, G. H. (Eds.). Silicon in agriculture. **Studies in plant science**, New York, v. 8, s/n., p. 85-113, 2001.

SANTOS, A. B.; SANTIAGO, C. M. **Informações técnicas para a cultura do arroz irrigado nas regiões Norte e Nordeste do Brasil**. Santo Antônio de Goiás, GO: Embrapa Arroz e Feijão, 2014. 150 p. (Documento Embrapa Arroz e Feijão, 279).

SANTOS, D. S. et al. Eficiência de fontes de silício para a cultura do arroz. **Enciclopédia Biosfera**, Goiânia, v. 6, n. 11, p. 1-10, 2010.

SANTOS, G. R. et al. Danos causados por doenças fúngicas no arroz cultivado em várzeas no Sul do estado do Tocantins. **Bragantia**, Campinas, v. 70, n. 4, p. 869-875, 2011.

SILVA-LOBO, V. L. et al. Influência da adubação nitrogenada, época de plantio e aerossposos sobre a severidade da mancha de grãos em arroz de terras altas. **Summa Phytopathologica**, Botucatu, v. 37, n. 3, p. 110-115, 2011.

SILVA, L. S.; BOHNEN, H. Produtividade e absorção de nutrientes pelo arroz cultivado em solução nutritiva com diferentes níveis de silício e cálcio. **Revista Brasileira Agrocência**, Pelotas, v. 9, n. 1, p. 49-52, 2003.

SOUZA JÚNIOR, I. T. et al. Biocontrole da queimada-bainhas e do nematoide-das-galhas e promoção de crescimento de plantas de arroz por rizobactérias. **Pesquisa Agropecuária Brasileira**, Brasília, v. 45, n. 11, p. 1259-1267, 2010.

TUMBARSKI, Y.; PETKOV, E.; DENKOVA, Z. Study on the influence of the cultural conditions and the composition of the culture medium on the antimicrobial activity of *Bacillus methylotrophicus* BM47 against some fungal phytopathogens. **Journal of Global Biosciences**, Washim, v. 4, n. 8, p. 2990-2996, 2015.

UTUMI, M. M. **Sistema de produção de arroz de terras altas**. 4. ed. Porto Velho, RO: Embrapa Rondônia, 2008. 33 p.