

Physiological performance of rice seeds treated with thiamethoxam or rhizobacteria under different temperatures¹

Vanessa Nogueira Soares^{2*}, Aline Klug Radke², Maria Ângela André Tillmann²,
Andréa Bittencourt Moura³, Luis Osmar Braga Schuch²

ABSTRACT - This study aimed to evaluate, at 15, 20 and 25 °C, the physiological potential and enzyme activity of seedlings from six samples of rice seeds stored for one year and treated with growth-promoting rhizobacteria (*Pseudomonas synxantha*) and the bioactivator thiametoxam. The following were evaluated: seed germination, first germination count, root protrusion speed index, emergence percentage and emergence speed index, shoot and root length and enzyme activity (esterases, α -amylase and peroxidases) at 15, 20 and 25 °C. In these conditions, thiametoxam and the rhizobacteria *P. synxantha* (DFs 185) improved the physiological performance of rice seeds with physiological qualities at low temperatures (20 and 15 °C) and did not change the expression of the esterase and peroxidase isoenzymes. The rhizobacteria *P. synxantha* (DFs 185) increased the expression of the enzyme α -amylase at low temperatures.

Index terms: *Oryza sativa* L., bioactivator, *Pseudomonas synxantha*, cold temperatures.

Desempenho fisiológico de sementes de arroz tratadas com rizobactérias e tiametoxam submetidas a diferentes temperaturas

RESUMO - O objetivo desse estudo foi avaliar nas temperaturas de 15, 20 e 25 °C o potencial fisiológico e a atividade enzimática de plântulas provenientes de seis amostras de sementes de arroz armazenadas por um ano, tratadas com rizobactéria promotora de crescimento (*Pseudomonas synxantha*) e bioativador tiametoxam. Foram avaliados: germinação, primeira contagem da germinação, velocidade de protrusão da raiz, percentual e índice de velocidade de emergência, comprimento das partes aérea e radicular e atividade enzimática (esterases, α -amilases e peroxidases) nas temperaturas de 15, 20 e 25 °C. Tiametoxam e a rizobactéria *P. synxantha* (DFs 185) melhoraram, nessa condição, o desempenho fisiológico de sementes de arroz de diferentes qualidades fisiológicas em baixa temperatura (20 e 15 °C) e não alteraram a expressão das isoenzimas esterase e peroxidase. A rizobactéria *P. synxantha* (DFs 185) aumentou a expressão da enzima α -amilase em baixas temperaturas.

Termos para indexação: *Oryza sativa* L., bioativador, *Pseudomonas synxantha*, frio.

Introduction

Cold stress is a major cause of yield reduction in rice crops. The occurrence of low temperatures at sowing time can damage crop establishment, reducing the initial stand and causing increased weed competition (Mertz et al., 2009).

Among rice-producing areas in Brazil, the state of Rio Grande do Sul is the most prone to damage as a result of low temperatures during the growing season. Germination, seedling emergence and especially the reproductive stage are more sensitive to cold stress (Pereira et al., 2005).

Plants respond to cold stress by means of molecular, cellular, and biochemical and physiological changes (Sanghera

et al., 2011). Selective breeding is aimed at resistance to low temperatures at germination and emergence in order to perform sowing earlier and prevent the reproductive phase from coinciding with the onset of cold weather (Mertz et al., 2009).

In several crops, including rice, studies (Almeida et al., 2013; Araujo et al., 2010; Acevedo et al., 2008; Ludwig and Moura, 2007) have been demonstrating the ability of microorganisms and bioactivators to increase the physiological potential of seeds microbiolized with bacteria or treated with the insecticide thiamethoxam.

Seed treatment with certain microorganisms are currently used in order to increase productivity, because they not only protect plants against the action of pathogens but also promote

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²Departamento de Fitotecnia, Universidade Federal de Pelotas, FAEM/UFPel, Caixa Postal 354, 96010-900 - Pelotas, RS, Brasil.

³Departamento de Fitossanidade, Universidade Federal de Pelotas, FAEM/UFPel, Caixa Postal 354, 96010-900 - Pelotas, RS, Brasil.

*Corresponding author <vnsoares@gmail.com>

growth. Such growth promotion refers to the development of plants in general, including beneficial effects on seed germination, emergence and seedling development, and production of grains and fruits (Kokalis-Burelle et al., 2006). Ludwig and Moura (2007) and Ludwig et al. (2009) identified the potential of *Pseudomonas synxantha* (Ehrenberg) Holland (DFs 185) for growth promotion in rice cultivation, which was applied to seeds via microbiolization of the latter.

Specific rhizobacteria can alter plant physiology by producing and/or releasing hormones and/or enzymes that regulate plant growth and development (Ryu et al., 2003). In this regard, Chen et al. (2000) observed that rhizobacteria were able to induce increased levels of enzymes such as peroxidases and polyphenol oxidases in roots of cucumber plants, while the bacteria *Bacillus subtilis* secrete important enzymes for plant nutrition (Powar and Jagannathan, 1982).

Thiamethoxam, used as bioactivator, is transported into the plant by cells through the activation of various physiological reactions such as protein expression. These proteins participate in various defense mechanisms and types of plant stress, allowing plants to handle adverse conditions better. Almeida et al. (2013) evaluated the influence of thiamethoxam on rice crops and the potential benefits of bioactivator on crops when seeds are kept under low temperatures during germination and emergence. They concluded that thiamethoxam applied to rice seeds at rates of 100 and 200 mL of product per 100 kg seed was effective in improving the physiological performance of rice seeds at temperatures between 13 and 25 °C.

Regarding the enzymatic effect in rice seeds, this bioactivator, at a rate of 100 mL per 100 kg seed, can increase the activity of α -amylase enzymes, facilitating the decomposition of starch and mobilization of reserves, as well as glucose 6-phosphate dehydrogenase and peroxidases, which act in the defense of plants exposed to environmental stress, facilitating crop establishment (Acevedo et al., 2008).

The aim of this study was to evaluate, at different temperatures, the physiological potential and the enzymatic expression of seedlings originated from rice seeds with different physiological qualities, treated with rhizobacteria and thiamethoxam.

Material and Methods

To evaluate the effect of the bioactivator and rhizobacteria on the physiological potential of rice seeds, an assessment was made of six samples of the cultivar BRS Querência with different physiological qualities, namely lots 2 and 6, both with 90% germination; lots 1, 3, 4 and 5 with 74, 82, 70 and 80% germination, respectively. The samples were stored for one year in multiwall paper packaging in a freezer.

The chemically treated seeds received thiamethoxam at a rate of 200 mL per 100 kg seed and were then stored. The product was applied directly on the bottom of a plastic bag before the seeds were put in. The seeds were then placed inside the bags and stirred until they were uniformly treated. A mixture volume (product + water) of 0.6 L was used for 100 kg seed.

As the tests were set up, the seeds treated with the bacterial isolate were microbiolized with suspension of strain DFs 185.

Selection of bacterial isolate: the isolate of *Pseudomonas synxantha* (Ehrenberg) Holland, identified as DFs 185, was pre-selected for the control of rice diseases and growth promotion by Ludwig and Moura (2007) and for growth promotion (germination and emergence) by Soares et al. (2012).

Seed microbiolization: the bacterial isolate was transferred to solid medium 523 (Kado and Heskett, 1970) and incubated at 28 °C for 24 h in BOD. From this culture, suspension was prepared in saline solution (0.85% NaCl), with concentration adjusted to $10^7 - 10^8$ UFC/mL ($A_{540}=0,5$). The seeds were immersed in the bacterial suspension and stirred for 30 minutes at 10 °C.

The control consisted of non microbiolized seeds that were not treated with thiamethoxam.

Germination: four sub-samples of 50 seeds were used, and the test was performed on towel paper according to the Rules for Seed Analysis (Brasil, 2009).

First germination count: performed under the same conditions of the germination test and assessed at seven days after sowing, following the recommendations of Brasil (2009).

Root protrusion speed: performed under the same conditions of the germination test, considering two mm of primary root as the beginning of visible germination. The assessment was performed daily with the aid of a millimeter ruler. Subsequently, these data were applied to the formula proposed by Edmond and Drapala (1958): $GS=(N1G1+N2G2+...+NnGn)/(G1+G2+...Gn)$,

where: GS is the germination speed in days; G1, G2, Gn is the number of germinated seeds at the first count, second count and last count; N1, N2, Nn is the number of days from sowing to first, second and last counts.

Emergence speed index (ESI) and emergence percentage: eight subsamples of 25 seeds were used; sowing was performed in sand in gerbox maintained under germination (25 and 20 °C) or in a growth chamber (15 °C) for 14 days; the number of seedlings emerged in each gerbox was counted daily. Data from the assessments were applied to the formula proposed by Maguire (1962) to get ESI: $ESI = E1/N1 + E2/N2 + ... + En/Nn$, where: ESI = emergence speed index; E1, E2, ... En = number of normal seedlings counted at the first count, second count and last count; N1, N2, ... Nn = the number of

days from sowing to first, second and last counts.

Seedling root and shoot length: assessment made under the same conditions of the germination test with four subsamples of ten seedlings; seeds were distributed at the upper third of the towel. The shoots and roots of normal seedlings were measured separately. The assessment took place after seven days with the aid of a millimeter ruler.

Enzyme activity: isozymes α -amylase, esterases and peroxidases were determined by electrophoresis, performed according to the methodology described by Mertz et al. (2009) for rice.

Experimental design: the study used a completely randomized factorial design 3 x 6 [(1 bacterial isolate + 1 thiamethoxam rate + control) and six samples] with four replicates for each temperature. Tukey's test was used for comparison of treatment means at 5% level of probability.

Results and Discussion

According to the results for germination and first germination count at 25 °C (Table 1), there was no positive effect of thiamethoxam and rhizobacteria (DFs 185) on germination of treated or microbiolized seeds. The percentage of first germination count for sample 4 (low germination) was impaired by the treatment with the bioactivator. The remaining samples did not differ statistically from control seeds. Araújo et al. (2010), when assessing the germination of rice seeds stored for one year and subsequently inoculated with strains of diazotrophic bacteria, found no positive effect of bacterial isolates, either. Dan et al. (2010) also had no positive results for germination and first germination count of soybean seeds treated with thiamethoxam.

Table 1. First germination count (FGC), germination (G) and root protrusion speed (RPS) at 25, 20 and 15 °C for samples of untreated rice seeds (US) and seeds treated with thiamethoxam (Thiam) or with bacterial isolate DFs 185 (*Pseudomonas synxantha*).

Sample	FGC (%)			G (%)			RPS (days)		
	US	Thiam	DFs 185	US	Thiam	DFs 185	US	Thiam	DFs 185
25 °C									
1	81 aA	72 abA	77 abA	84 aA	80 aA	81 aA	2.90 aA	2.86 aA	2.68 aA
2	78 aA	76 abA	82 aA	82 abA	87 aA	83 aA	2.89 aA	2.97 aA	2.69 aA
3	54 bA	64 bA	61 bA	74 bA	67 bA	70 bA	2.96 aA	3.00 aA	2.62 aA
4	28 cA	13 cB	24 cAB	45 cA	41 cA	44 cA	4.25 bA	3.89 bA	3.99 bA
5	80 aA	73 abA	81 aA	82 abA	80 aA	87 aA	2.48 aA	2.83 aA	2.76 aA
6	73 aA	81 aA	76 abA	85 aA	86 aA	83 aA	2.55 aA	2.74 aA	2.48 aA
CV=7.96 %			CV=4.4 %			CV=11.59 %			
20 °C									
Sample	US	Thiam	DFs 185	US	Thiam	DFs 185	US	Thiam	DFs 185
1	45 cB	56 aA	57 aA	56 Bb	76 aA	74 aA	3.29 aA	3.52 bA	3.33 aA
2	67 aA	58 aB	58 aB	73 aA	76 aA	72 abA	3.03 aA	3.21 bA	3.34 aA
3	47 cA	36 bB	42 bAB	55 bB	57 bAB	64 bA	3.29 aA	3.29 bA	3.59 aA
4	6 dA	4 cA	4 cA	16 cA	13 cA	16 cA	3.50 bA	4.50 aB	4.58 bB
5	55 bcAB	61 aA	51 abB	73 aA	68 aAB	64 bB	3.04 aA	3.11 bA	3.17 aA
6	61 abA	58 aA	61 aA	66 aB	72 aAB	77 aA	3.18 aA	3.24 bA	3.19 aA
CV=6.19 %			CV= 4.61 %			CV=6.61 %			
15 °C									
Sample				US	Thiam	DFs 185	US	Thiam	DFs 185
1				14 cC	33 aA	20 bB	5.96 cB	5.96 cB	4.75 aA
2				31 aA	19 bcB	14 bB	5.86 bcB	5.82 abB	5.26 bA
3				19 bcA	13 cB	17 bAB	5.52 aB	5.70 aB	5.24 bA
4				2 dA	1 dA	2 cA	0.00 dA	0.00 eA	0.00 eA
5				24 abA	22 bA	21 bA	5.68 abA	5.95 cB	5.78 cAB
6				21 bB	22 bB	40 aA	5.81 bcA	6.20 dB	6.14 dB
				CV=8.79 %			CV=2.13 %		

*Means followed by the same lowercase letter in the column and capital letter in line, at each temperature and assessment, do not differ by Tukey's test at 5% probability. CV= Coefficient of variation.

At 20 °C, thiamethoxam and the rhizobacteria (DFs 185) produced a positive effect in sample 1; there was better performance of 20 and 18 percentage points, respectively, for the two assessments, compared to untreated seeds. However, microbiolization with the rhizobacteria (DFs 185) and treatment with the bioactivator resulted in reduced germination percentage and first germination count for sample 2, and thiamethoxam produced the same effect for sample 3 (Table 1). For samples 3 and 6, microbiolization with the rhizobacteria (DFs 185) gave a higher germination percentage, nine and 11 percentage points, respectively.

In this study, the assessment of first germination count at 15 °C was discarded because germination was impaired by the low temperature. Germination percentage at 15 °C for sample 1 (Table 1) was higher in seeds treated with thiamethoxam and the rhizobacteria (DFs 185). Treatment with the former was more effective than with the latter. However, for sample 6, only the rhizobacteria (DFs 185) showed a positive effect compared to untreated seeds. The germination of samples 2 and 3 was impaired by the bioactivator.

Low germination at 15 °C can be explained by the fact that the ideal temperature for the development of rice ranges between 25 and 30 °C (Yoshida, 1981). Temperatures below this range may cause cold stress, which is considered one of the most important abiotic stress for rice (Mertz et al., 2009). According to Yoshida (1981), damage such as failure in germination, delayed emergence and reduced plant height are common as a result of cold weather.

Data from Table 1 show that there was no statistical difference between treatments for germination speed, as assessed by root protrusion at 25 and 20 °C, except for sample 4 at 20 °C, whose germination rate (measured in days) increased because of the treatment with the bioactivator and microbiolization with the rhizobacteria (DFs 185). At 15 °C, the germination speed of samples 1, 2 and 3, in seeds treated with the rhizobacteria (DFs 185) was reduced, which resulted in less time (days) for root protrusion (Table 1). Germination speed was higher, resulting in an increase in the number of days for root protrusion to occur for samples 5 and 6, treated with thiamethoxam, and sample 6, microbiolized with the rhizobacteria (DFs 185) (Table 1).

The emergence speed index (ESI) at 25 °C, according to Table 2, had little variation between treatments. A significant increase was observed for sample 1 with thiamethoxam compared to control; however, the result was similar to the one obtained with the rhizobacteria (DFs 185). A significant reduction was observed in ESI in samples 2 and 4 when they were treated with thiamethoxam or microbiolized

with the rhizobacteria (DFs 185). At 20 °C, it was found that the sample with the lowest quality (sample 4) showed lower ESI when treated with thiamethoxam. For the other samples, there was no difference between untreated seeds and seeds microbiolized with the rhizobacteria (DFs 185) or seeds treated with thiamethoxam (Table 2). According to Dan et al. (2010), seedlings with greater ESI have better performance and, thus, greater ability to tolerate stresses that may affect plant growth and development. It is not unusual to observe variable behavior between different samples of seeds treated with microorganisms that promote plant growth and/or biocontrol. Corrêa et al. (2008) assessed the growth-promoting capacity of some rhizobacteria and noted that some treatments allowed an increase in the germination percentage of bean seeds, although more intense effects have been observed for ESI, especially in sample B. Nevertheless, the assessments of root dry weight and leaf dry weight had higher results in sample A for plants measured both individually and together.

Table 2 also shows that the emergence percentage at 25 °C for samples 1 and 6 was higher for seeds treated with thiamethoxam or microbiolized with the rhizobacteria (DFs 185), but for sample 3, only the rhizobacteria (DFs 185) led to the emergence of the sample, unlike the mean of the untreated seeds. However, for samples 2 and 4, there was a decrease in the emergence percentage of seeds treated with thiamethoxam and microbiolized with the rhizobacteria (DFs 185). For the same variable at 20 °C, sample 4 showed the lowest emergence percentage for control seeds and seeds treated with thiamethoxam compared with seeds microbiolized with rhizobacteria (DFs 185). Kloepper et al. (1986) observed increased seedling emergence up to 100%, caused by rhizobacteria of the genus *Pseudomonas*, when they conducted experiments in the laboratory and in the field. This unstable behavior of the rhizobacteria (DFs 185 - *P. synxantha*) is often observed for the biocontrol of plant diseases and attributed to the difference in the microorganism's capacity to colonize host tissues (Schisler et al, 2000.). Therefore, the same may happen for growth promotion, particularly the germination and emergence variables.

Table 3 shows that there was no effect of the bioactivator and the bacterial isolate on seedling shoot length at 25 °C. At 20 °C, only the rhizobacteria (DFs 185) increased seedling shoot length in sample 3 (Table 3). In the same table, a reduction can be seen in seedling root length at 25 °C for samples 1, 5 and 6 treated with thiamethoxam and for samples 1 and 6 microbiolized with the rhizobacteria (DFs 185). However, this effect did not occur at 20 °C, a temperature at which there was no difference between treatments (Table 3).

Table 2. Emergence speed index (ESI) and emergence percentage at 25 and 20 °C of samples of untreated rice seeds (US) and rice seeds treated with thiamethoxam (Thiam) or with bacterial isolate DFs 185 (*Pseudomonas synxantha*) at 14 days.

Sample	ESI			Emergence (%)		
	25 °C					
	US	Thiam	DFs 185	US	Thiam	DFs 185
1	9.12 bcB	10.21 aA	10.15 aAB	78 bC	91 aA	86 aB
2	10.75 aA	9.16 abB	9.26 aB	87 aA	82 bB	81 bB
3	7.80 bcA	6.96 cA	7.02 bA	58 cA	60 cA	64 cA
4	2.31 dA	0.83 dB	0.94 cB	29 dA	13 dB	14 dB
5	8.01 bcA	8.74 bA	8.96 aA	79 bA	82 bA	82 abA
6	9.32 bA	9.58 abA	9.51 aA	82 bB	88 aA	86 aA
	CV=7.01 %			CV=2.68 %		
Sample	20 °C					
	US	Thiam	DFs 185	US	Thiam	DFs 185
	1	4.96 aA	4.39 aA	4.86 aA	68 abA	74 aA
2	5.24 aA	5.09 aA	4.37 aA	66 aA	65 aA	74 abA
3	2.89 abA	3.67 aA	2.42 aA	48 aA	51 aA	52 bA
4	0.80 bAB	0.41 bB	2.44 aA	15 bB	11 bB	19 bA
5	5.27 aA	5.30 aA	4.87 aA	76 aA	76 aA	75 aA
6	4.73 aA	4.39 aA	4.18 aA	67 aA	62 aA	69 abA
	CV=25.29 %			CV=14.91 %		

*Means followed by the same lowercase letter in the column and capital letter in line, at each temperature and assessment, do not differ by Tukey's test at 5% probability.

CV= Coefficient of variation.

Table 3. Shoot length (SL) and root length (RL) of rice seedlings derived from samples of untreated rice seeds (US) and rice seeds treated with thiamethoxam (Thiam) or with bacterial isolate DFs 185 (*Pseudomonas synxantha*) at 25 and 20 °C at 7 days on paper roll.

Sample	SL (cm)			RL (cm)		
	25 °C					
	US	Thiam	DFs 185	US	Thiam	DFs 185
1	4.27 aA	4.07 aA	3.53 bA	8.20 aA	4.60 aB	4.33 cB
2	4.40 aA	3.57 aA	4.47 abA	5.37 bAB	4.33 aB	6.20 bcA
3	4.30 aA	4.70 aA	4.57 abA	7.47 abA	6.07 aA	6.73 bA
4	4.13 aA	3.63 aA	3.60 bA	7.47 abA	4.53 aA	6.10 bcA
5	3.97 aA	4.63 aA	5.03 aA	7.47 abA	6.30 aB	9.17 aA
6	4.17 aA	3.47 aA	4.10 abA	7.47 abA	5.93 aB	6.17 bcB
	CV=14 %			CV=14.02 %		
Sample	20 °C					
	US	Thiam	DFs 185	US	Thiam	DFs 185
	1	1.73 aAB	1.60 aB	1.97 aA	3.10 aA	3.33 aA
2	1.66 aB	1.86 aAB	2.00 aA	3.00 aA	3.33 aA	3.53 aA
3	1.56 aA	1.67 aAB	1.57 aA	3.17 aA	3.63 aA	3.47 aA
4	1.10 bA	0.87 bA	0.97 cA	1.60 aA	2.07 aA	1.90 bA
5	1.73 aA	1.67 aA	1.70 abA	3.00 aA	3.33 bA	3.10 aA
6	1.66 aA	1.86 aA	1.77 abA	3.20 bA	3.57 aA	3.37 aA
	CV=8.74 %			CV=10.53 %		

*Means followed by the same lowercase letter in the column and capital letter in line, at each temperature and assessment, do not differ by Tukey's test at 5% probability.

CV= Coefficient of variation.

Shoot and root lengths at 15 °C could not be assessed because at seven days after the onset of testing, the seeds had not germinated yet.

As shown in Figure 1, as for the expression analysis of the enzyme amylase, it was observed that at 25 °C there was no difference between seedlings originated from seeds microbiolized with the rhizobacteria (DFs 185) or seeds treated with thiamethoxam, probably due to favorable conditions for germination.

However, at 20 and 15 °C, increased enzyme activity was observed in some treatments in seedlings originated from seeds microbiolized with the rhizobacteria *P. synxantha* (DFs 185). Markovitz and Klein (1955) reported the production of α -amylase by bacteria of the genus *Pseudomonas*. However, Freitas and Aguilar Vildoso (2004) reported unstable behavior of some isolates of *Pseudomonas* in promoting plant growth. The authors attribute such behavior to the susceptibility of the bacteria of this genus to the medium and/or a very specific mode of action.

In the esterase isozyme system at 25 °C (Figure 2), the

presence of six alleles (EST 1, EST 2, EST 3, EST 4, and EST 5, EST 6) was identified. However, at 20 °C and 15 °C, four alleles were observed (EST 1, EST 2, EST 3 and EST 4). This is indicative of the influence of temperature and shows that the bioactivator and the rhizobacteria (DFs 185) had no effect on enzyme expression. This fact can be explained by the involvement of esterases in the hydrolysis of esters; therefore, the former are directly associated with lipid metabolism, which is faster at optimum temperature. These results are in agreement with those of Mertz et al. (2009), who evaluated physiological changes in rice seeds exposed to cold temperatures in the germination phase. They reported that low temperatures caused a reduction in the expression of the esterase enzyme.

The isozyme profile of peroxidase (Figure 3) did not enable the observation of differences in the expression and intensity of the bands that could be attributed to thiamethoxam or the rhizobacteria (DFs 185). At 25 and 15 °C, the electrophoretic patterns presented four alleles (PO1, PO2, PO3 and PO4), but the presence of three alleles was observed at 20 °C.

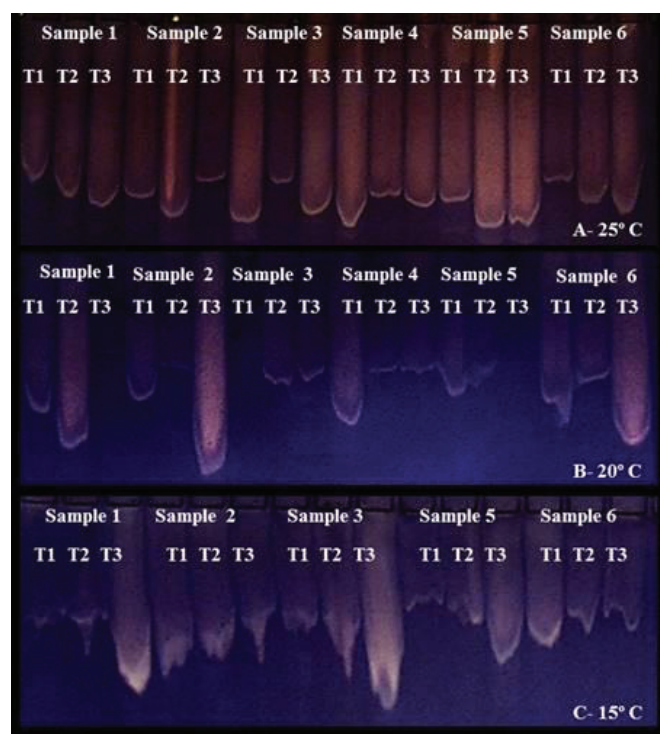


Figure 1. Electrophoretic patterns of the enzyme α -amylase in rice seedlings of six untreated samples (T1), samples treated with thiamethoxam (T2) or with *Pseudomonas synxantha* (T3) at five days (25 and 20 °C) and 14 days (15 °C). A- 25 °C; B- 20 °C; C- 15 °C.

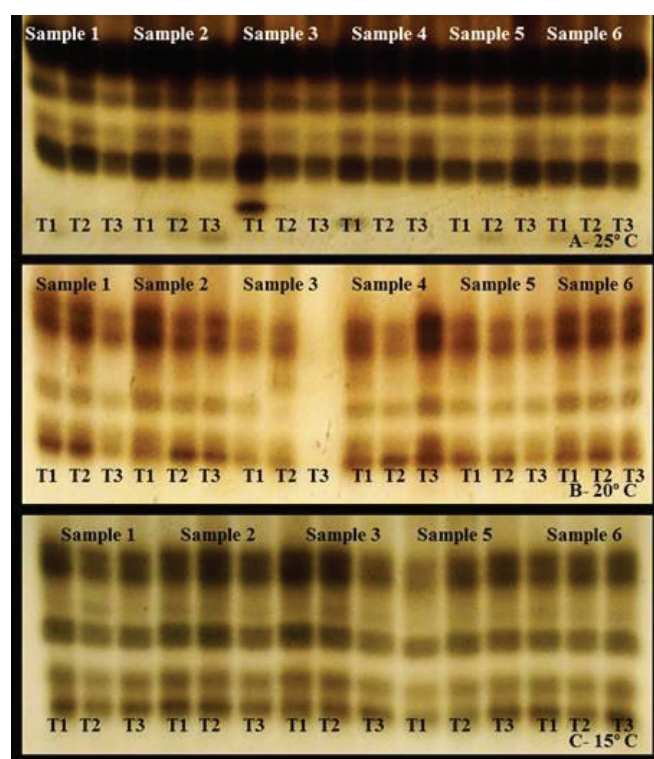


Figure 2. Electrophoretic patterns of the esterase enzyme in rice seedlings of six untreated samples (T1), samples treated with thiamethoxam (T2) or with *Pseudomonas synxantha* (T3), at five days (25 and 20 °C) and 14 days (15 °C). A-25 °C; B-20 °C; C-15 °C.

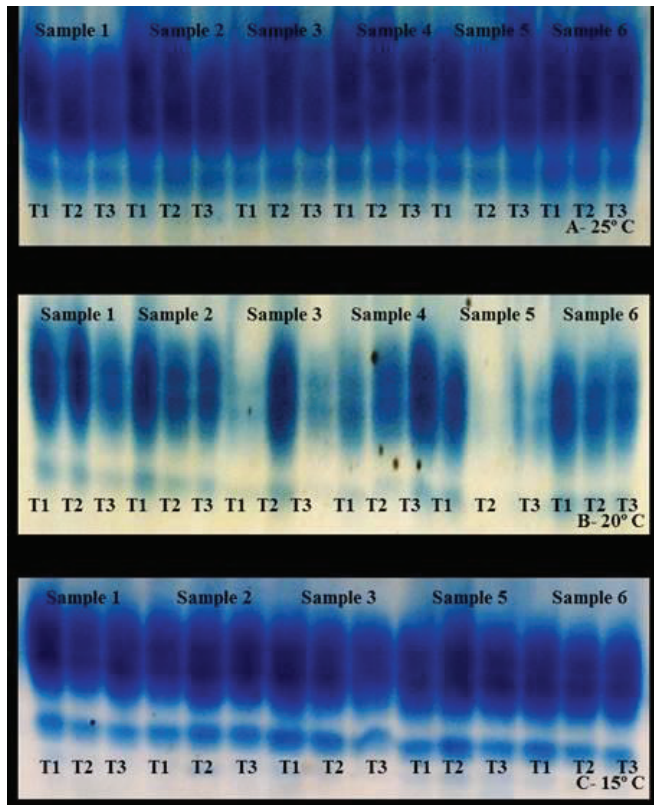


Figure 3. Electrophoretic patterns of the peroxidase enzyme in rice seedlings of six untreated samples (T1), samples treated with thiamethoxam (T2) and *Pseudomonas synxantha* (T3) at five days (25 and 20 °C) and 14 days (15 °C). A-25 °C; B-20 °C; C-15 °C.

Peroxidases are isoenzymes associated with changes in the physiological processes of plants under stress. The presence of four alleles at 25 °C can be associated with the germination process, which was faster at a favorable temperature than at 20 °C. However, there was greater expression and intensity of the enzyme at 15 °C, possibly because of stress caused by the low temperature. According to Vieira et al. (2008), the effect of peroxidases on seed germination is due to both the beneficial oxidative action of phenolic compounds, which may be natural germination inhibitors, and the competition for the oxygen available, in this case, delaying or inhibiting germination. The role of peroxidases is associated with cell wall lignification, oxidation of indole acetic acid, ethylene and participation in the seed dormancy process; in some cases, their effect may be enhanced if it is associated with biotic and abiotic factors, and serves as biochemical marker of stress (Menezes et al., 2004).

The absence of seed germination in sample 4 at 15 °C prevented the electrophoretic assessment of isoenzymes.

The different behavior of the treatments in the assessed

samples of rice seeds can be explained in different ways. In the case of the bioactivator thiamethoxam, the results may have been influenced by the storage of treated seeds, as reported by Dan et al. (2010). They recommend that seeds should be treated with thiamethoxam close to the time of sowing, because a reduction of physiological quality of soybean seeds was observed when the seeds treated with the bioactivator had been stored for longer. Piccinin et al. (2013) also concluded that thiamethoxam adversely affects the physiological quality of soybean seeds that were treated and stored. Rosa et al. (2012) also found a similar result when evaluating the physiological quality of seeds of hybrid maize treated with thiamethoxam and stored. Regarding the rhizobacteria, the results may vary due to the ability of the microorganism to colonize the host tissue (Schisler et al., 2000). Seed colonization depends on several factors, such as temperature, amount and composition of exudates, incidence of microorganisms antagonistic to rhizobacteria on seeds. As mentioned by Freitas and Aguilar Vildoso (2004), the group of fluorescent *Pseudomonas* may present inconsistent results in promoting plant growth, a fact that occurred in this study. The susceptibility of bacteria to the medium and their very specific mode of action may be the responses so that changes in environmental conditions can alter the capacity or intensity of the bacteria to promote plant growth, especially if this capacity depends on a metabolic pathway that may result in the production of a single substance which is responsible for the beneficial effect on the plant (Freitas and Aguilar Vildoso, 2004). In this sense, knowledge of the mode of action of growth-promoting rhizobacteria is essential so that such information can guide the choice of the best way to use these microorganisms.

Conclusions

Thiamethoxam and the rhizobacteria *P. synxantha* (DFs 185) improve the physiological performance of rice seeds of different physiological qualities at low temperature (20 and 15 °C) and do not alter the expression of esterase and peroxidase isozymes. The rhizobacteria *P. synxantha* (DFs 185) increase the expression of the enzyme α -amylase at low temperatures.

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