# RESEARCH NOTE

# Treatment of rice seeds with thiamethoxam: reflections on physiological performance<sup>1</sup>

Andreia da Silva Almeida<sup>2</sup>, Cristiane Deuner<sup>2</sup>, Carolina Terra Borges<sup>2\*</sup>, Géri Eduardo Meneghello<sup>2</sup>, Adilson Jauer<sup>3</sup>, Francisco Amaral Villela<sup>2</sup>

ABSTRACT - Thiamethoxam is a systemic insecticide that is transported within the plant through its cells and can activate various physiological reactions such as protein expression. These proteins interact with defense mechanisms against stress in adverse growing conditions. The objective of this study was to evaluate the effect of thiamethoxam in rice seeds and the potential benefits that it can provide. Two experiments were carried out and, in both, seeds were treated with commercial product containing 350 g of thiamethoxam active ingredient per liter of product, at doses 0, 100, 200, 300 and 400 mL.100 kg<sup>-1</sup> of seeds: 1) it was conducted with three lots of IRGA BR 424 cultivar rice seeds, which were submitted to the following laboratory tests: germination, cold test, accelerated aging test, as well as field assessment: total seedling length, root system length, number of panicles and productivity; 2) four lots of IRGA BR 424 cultivar rice seeds, two high and two low-vigor, were subjected to the following tests: germination, cold test and greenhouse seedling emergence test. Thiamethoxam rice seed treatment positively favors the seed quality.

Index terms: Oryza sativa, bioactivator, vigor.

# Potencial fisiológico de sementes de arroz tratadas com tiametoxam

RESUMO - O tiametoxam é um inseticida sistêmico que é transportado dentro da planta através de suas células e pode ativar várias reações fisiológicas como a expressão de proteínas. Estas proteínas interagem com mecanismos de defesa a estresses em condições adversas de cultivo. O objetivo deste trabalho foi avaliar o efeito do tiametoxam em sementes de arroz e os potenciais benefícios que o tratamento possa proporcionar. Foram realizados dois experimentos, sendo em ambos as sementes tratadas com produto comercial contendo 350 gramas de ingrediente ativo de tiametoxam por litro nas concentrações de 0, 100, 200, 300 e 400 mL. 100 kg<sup>-1</sup> de sementes: 1) realizado com três lotes de sementes de arroz, da cultivar IRGA BR 424, as quais foram submetidas a testes no laboratório: germinação, teste de frio, envelhecimento acelerado, e avaliações à campo: comprimento total de plântula e radicular, número de panículas e produtividade; 2) utilizados quatro lotes de sementes da cultivar IRGA BR 424, sendo dois de alto vigor e dois de baixo, submetidos aos seguintes testes: germinação, teste de frio e emergência em casa de vegetação. O tratamento de sementes de arroz com tiametoxam favorece o desempenho ou potencial fisiológico das sementes.

Termos para indexação: Oryza sativa, bioativador, vigor.

### Introduction

In Brazil, rice is the third most important agricultural product after soybeans and corn (Mielezrski et al., 2008), and the State of Rio Grande do Sul is the largest producer with 1,0666 million hectares, representing 44.5% of the national territory and 67.0% of Brazilian production (CONAB, 2013).

A set of techniques contribute towards achieving this productivity, among them, the use of agrochemicals such as fungicides, herbicides and insecticides. However, some insecticides may induce effects that are still unknown, modifying plant metabolism and morphology, with particular reference to physiological and morphological changes in plants caused by carbofuran (Freitas et al., 2001) and

<sup>&</sup>lt;sup>1</sup>Submitted on 03/06/2014. Accepted for publication on 05/29/2014. <sup>2</sup>Departamento de Fitotecnia, UFPel, Caixa Postal 354, 96010-900 - Capão do Leão, RS, Brasil.

<sup>&</sup>lt;sup>3</sup>Syngenta Proteção de Cultivos LTDA, 86010-020 - Londrina, PR, Brasil. \*Corresponding author <carol\_tborges@hotmail.com>

thiamethoxam (Calafiori and Barbieri, 2001). Some of these metabolic alterations allow us to classify the latter as a product with a bioactivating ability.

Bioactivators are complex organic substances, which modify plant growth and are capable of acting on the DNA transcription into the plant, gene expression, membrane proteins, metabolic enzymes and mineral nutrition (Castro and Pereira, 2008). The insecticide thiamethoxam has shown positive effects like vigor expression increase, biomass accumulation, high photosynthetic rate and deeper roots in soybean (Castro et al., 2007) and rice culture (Almeida et al., 2011).

Thiamethoxam is transported into the plant by its cells and activates several physiological reactions such as protein expression. These proteins interact with various plant defense mechanisms against stress, preparing to face up biotic and abiotic adversities such as drought, low pH, high soil salinity, free radicals, high temperature stress, toxic effects due to high levels of aluminum, injuries caused by pests, wind, hail, viruses and nutrient deficiencies. It has a phytotonic effect, i.e., it helps the plants develop quickly, boosting vigor. It has been associated with increase in the vigor, leaf and radicular area index, a more uniform stand, emergence uniformity and better initial development in soybean (Castro and Pereira, 2008), rice (Almeida et al., 2011), carrot (Almeida et al., 2009), cotton (Lauxen et al., 2010) and black oat (Almeida et al., 2012).

An efficient use of thiamethoxam can be applied for seed treatment, as it accelerates the germination process, inducing a further development of the embryonic axis and minimizing negative effects in situations of presence of aluminum, salinity and low water supply (Cataneo, 2008). This author has also observed a reduction in the time required for soybean field establishment, decreasing the negative impact of weed competition or competition for essential nutrients present in the soil.

In this context, the aim of the present study was to evaluate the effect of thiamethoxam in rice seeds and the potential benefits that this treatment can offer.

# **Material and Methods**

All experiments were conducted at the Didactic Laboratory of Seed Analysis (LDAS), Eliseu Maciel Faculty of Agronomy, Federal University of Pelotas (UFPel), and at an experimental area within a rural property located in Dom Pedrito - RS. The seeds were treated with a commercial product containing 350 g of thiamethoxam active ingredient per liter of product. Five doses were used: zero (untreated); 100, 200, 300 and 400 mL.100 kg<sup>-1</sup> of seeds prior to sowing. The solution volume used was 0.6 L.100 kg<sup>-1</sup> of seeds.

The present work was divided into two different

experimental procedures as described below.

#### Experiment 1

This experiment was divided into two stages: the first was carried out right after the treatment at the LDAS, and the second stage was conducted at the experimental area in Dom Pedrito. Experimental materials used were: three lots of IRGA BR 424 cultivar rice seeds, and the following evaluations were conducted:

*Germination*: we used four replicates, with four subsamples of 50 seeds for each treatment; the seeds were sown in paper rolls moistened with distilled water equivalent to 2.5 times the weight of dry paper and kept in a germination chamber at 25 °C. Evaluations were performed on day 5 and 14 after sowing (Brasil, 2009).

*Cold test*: we used four replicates of 50 seeds for each treatment. The seeds were sown in paper rolls imbibed in distilled water equivalent to 2.5 times the weight of dry paper, kept for seven days in a refrigerator at 10 °C; and then placed in a germination chamber at 25 °C. Evaluations were performed according to Brasil (2009). The counting of normal seedlings was performed five days after test installation and results were expressed as percentage of normal seedlings;

Accelerated ageing test: carried out using the plastic box method (McDonald Jr. and Phaneendranath, 1978), in which seeds are distributed in a single layer on the inner screen and on the bottom containing 40 mL of distilled water. The boxes containing the seeds were incubated at 42 °C for 120 hours in BOD. After the aging period, the seeds were sown according to the methodology of the germination test. The percentage of normal seedlings was determined at day 5.

Parameters evaluated in the experimental area:

*Total seedling length*: ten replicates of 10 seedlings were used for each treatment. The total length of the seedling was measured at day 21 after sowing and results were expressed in cm per plant;

*Root system length*: ten replicates of 10 seedlings were used for each treatment. The length of the root system was measured at day 21 after sowing and results were expressed in cm per plant;

In order to evaluate the number of panicles and productivity, sowing was done in plots of 3 x 5 meters, at a density of 110 kg.ha<sup>-1</sup>. The crop management was performed according to the technical recommendations for irrigated rice. To obtain the number of panicles per m<sup>2</sup>, an area of 1 m<sup>2</sup> (1 x 1) was demarcated within the plot where panicles were hand cut. After counting, those panicles were tracked and then weighed to obtain the yield.

We used a completely randomized design for the experiment, involving a  $3 \times 5$  factorial (3 lots x 5 doses of the product) and four replicates for each lot, separately. For field evaluation (total seedling length, root length, panicle number and

productivity), we divided the scheme in portions, considering each lot as a portion. Results obtained were subjected to analysis of variance, and data obtained on dose effects were used for polynomial regression with p <0.05 considered a significant level, using the statistical program WinStat 1.0 (Machado and Conceição, 2003). Data with values expressed in percentage were subjected to arcsine transformation (root x /100).

## Experiment 2

IRGA BR 424 cultivar rice seeds, two with higher vigor and two with lower vigor, were used. The parameters evaluated were: germination, cold test (both mentioned in experiment 1) and greenhouse seedling emergence test: four replications were sown, each one consisting of four subsamples of 50 seeds per lot, in plastic trays containing a 2 cm deep layer of sand. Irrigation took place whenever it was necessary, and the evaluation was carried out at day 15 after sowing, when seedling emergence became constant; and thus the percentage of normal seedlings emerged was registered (Nakagawa, 1999).

We used a completely randomized design for the

experiment, involving a  $4 \ge 5$  factorial (4 lots  $\ge 5$  doses of the product), and four replicates for each lot, separately.

# **Results and Discussion**

#### Experiment 1

It was observed that the three rice seeds lots treated with thiamethoxam showed superior performance in all characteristics studied in comparison with control treatment values (without application of thiamethoxam), varying only the intensity of this difference due to the dose. However, at higher doses, we verify a reduction in the values observed at the point of maximum technical efficiency, probably because of the product's phytotoxic effect (Figures 1 and 2).

The application of the product in the lots of rice seeds used (Figure 1A) showed that from zero dose, germination has and increasing trend, reaching a peak when the dose varies from 100 a 200 mL.100 kg<sup>-1</sup> of seeds. After reaching this point, germination decreases with an increase in the dose of the product.

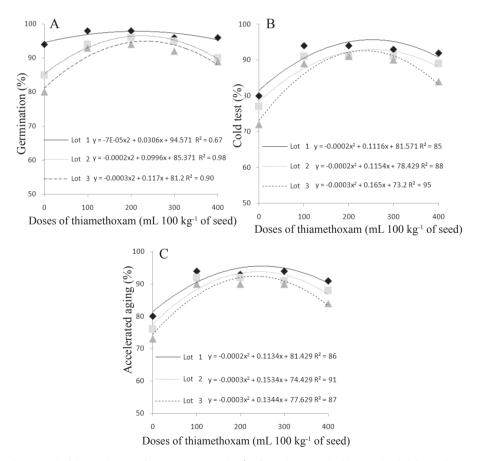


Figure 1. A - Germination (%)/ Thiamethoxan doses (mL.100 kg<sup>-1</sup> of seeds); B - Cold test (%)/Thiamethoxan doses (mL.100 kg<sup>-1</sup> of seeds); C - Accelerated Aging Test (%)/Thiamethoxan doses (mL.100 kg<sup>-1</sup> of seeds) of three lots of IRGA BR 424 cultivar rice seeds.

In lots 2 and 3, an increased germination occured in comparison with lot 1, however, the three lots have shown significant differences. Results of the present study confirm those obtained by Almeida et al. (2009) working with carrot; Almeida et al. (2011) with rice; Clavijo (2008) with rice; Castro and Pereira (2008) with soybean. Authors unanimously maintain that seeds treated with thiamethoxam demonstrated accelerated germination rates due to the stimulation of enzymatic activity, in addition, they exhibited a more uniform emergence and stand, and a better initial development. Also, in soybean seeds, Cataneo (2008) observed that thiamethoxam accelerates germination, induces further development of the embryonic axis, minimizing the negative effects caused by stressful situations.

Seeds treated with thiamethoxam showed an increase in the percentage of normal seedlings in cold test (Figure 1B) compared to seeds that did not receive the product (zero dose). There was a variation in behavior, but dose-response curves for the three seed lots tested have shown a similar trend, differing only in the lot 3, which stood out from the others displaying a sharper curve.

As in previous tests, we can see that from zero dose, the response curve follows a further upward trend, reaching a peak at doses around 200 mL.100 kg<sup>-1</sup> of seeds. This better resistance occurs probably due to the fact that thiamethoxam

moves through plant cells and activates several physiological reactions, such as the expression of functional proteins related to defense mechanisms of plants against stress factors such as drought, high temperatures, toxic effects among others, improving productivity, foliar and root area, as found in a study with soybean seeds (Tavares et al., 2008).

The germination process of the seeds treated with thiamethoxam after the accelerated aging test showed significant differences from the zero dose (Figure 1C). These results are in agreement with those obtained by Almeida et al. (2011) in rice cultivars. The increase that occurred at the cold test and at the accelerated aging test, from zero dose to 200 mL.100 kg<sup>-1</sup> of seeds, corresponds to the maximum point and is higher than that observed in germination.

As shown in Figure 2A, rice seeds treated with thiamethoxam showed an increase in the total seedling length compared with untreated controls, reaching a peak between doses 200 and 300 mL.100 kg<sup>-1</sup> of seeds in lots 2 and 3. At higher doses, a decreasing trend in total seedling length occurs, which can be clearly seen in lot 2. Lot 1 seedlings showed more a uniform length compared with those from the other lots, and, on its turn, in lot 2, the dose 100 mL considerably increased the length of the seedlings compared with those that did not receive thiamethoxam.

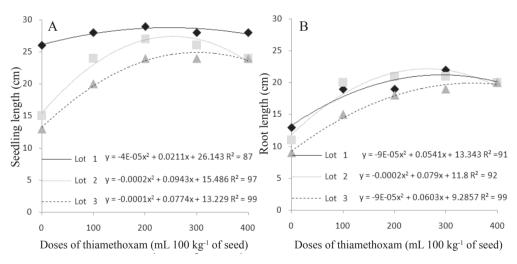


Figure 2. A - Seedling length (cm)/ Thiamethoxan doses (mL.100 kg<sup>-1</sup> of seeds); B - Root length (cm)/ Thiamethoxan doses (mL.100 kg<sup>-1</sup> of seeds) of three lots of IRGA BR 424 cultivar rice seeds.

This difference between zero dose and the dose that showed higher response can be explained by the hypothesis that thiamethoxam favors water uptake and stomatal resistance, improving the water balance of plant (Castro et al., 2007). As observed in soybean (Tavares et al., 2008), carrot (Almeida et al., 2009) and rice (Almeida et al., 2011), root development increases the absorption of nutrients, increasing leaf area and the expression of plant vigor.

In a similar manner to seedling length, seeds subjected to treatment with thiamethoxam showed increases in root length (Figure 2B). The curves displayed a peak at doses between 200 and 400 mL.100 kg<sup>-1</sup> of seeds, depending on the lot. These results corroborate those of Almeida et al. (2011), working with rice, and Lauxen et al. (2010), with cotton. Castro and Pereira (2008) observed higher levels of macro and micronutrients in plant tissue, increased productivity and elongation of the main root in soybean seedlings grown from seeds treated with thiamethoxam. In contrast, Puccinin et al. (2013) studied the influence of the insecticides fipronil and thiamethoxam in physiological performance of soybeans seeds stored, and they have found that insecticides after the storage period adversely affect the root length.

Field assessment revealed that the number of panicles

per m<sup>2</sup> reached a maximum point at dose 200 mL.100 kg<sup>-1</sup> of seeds, displaying an increase of approximately 140 panicles/ m<sup>2</sup> (Figure 3A). As for productivity results (Figure 3B), an increase between 1000 and 2000 kg.ha<sup>-1</sup> at the maximum efficiency dose was observed. Damico (2008) evaluated the application of thiamethoxam in seeds of two soybean cultivars and found that there was no difference between treatments on plant population and on stem diameter, however, grain yields were higher in seeds treated with thiamethoxam.

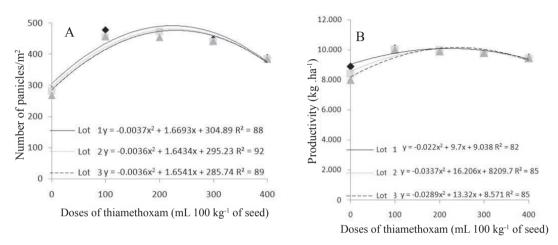


Figure 3. A - Number of panicles/m<sup>2</sup> / Thiamethoxan doses (mL.100 kg<sup>-1</sup> of seeds); B - Productivity (kg.ha<sup>-1</sup>)/ Thiamethoxan doses (mL.100 kg<sup>-1</sup> of seeds) of three lots of IRGA BR 424 cultivar rice seeds.

#### **Experiment** 2

As for germination test results (Figure 4A), one can verify that lower vigor seeds, lots 3 and 4, had higher increases in germination rate compared with that from the control. In lots 1 and 2 this rate of increase was also larger than at zero dose, but this increase was at a lower index in comparison to lower vigor lots. The results of the present study confirm those of Castro et al. (2007), working with soybean, Clavijo (2008) with rice and Almeida et al. (2012) with black oat, stating that seed treated with thiamethoxam had an accelerated germination process due to the stimulation of enzymatic activity, besides showing a more uniform emergence and stand, and a better initial development.

As shown in Figure 4B, lots 3 and 4 exhibited a larger increase at dose 200 mL in comparison to that obtained at zero dose in the cold test. Thiamethoxam insecticide has shown those positive effects such as an increased expression of vigor, phytomass accumulation, a high photosynthetic rate and deeper roots (Castro and Pereira, 2008).

In accordance with other variables analyzed in this

experiment, in the greenhouse seedling emergence test, lots 1 and 2 showed significant increases, with the greatest results found at dose 200 mL dose in relation to zero dose, however this increase, using the same dose, was lower than that one obtained in lots 3 and 4 (Figure 4C). Thiamethoxam activates various physiological reactions, such as the expression of functional proteins related to defense mechanisms of plants against stress factors such as drought, high temperatures, toxic effects, among others, improving productivity, foliar and root areas (Tavares et al., 2007).

The beneficial effects of the product do not depend on the physiological quality of the evaluated lots, but they were more evident in lots 3 and 4 (lower vigor) than in lots 1 and 2 (higher vigor).

Thiamethoxam is very important for rice cultivation, considering that, in field conditions, germination process may be low, slow, irregular, with an uneven emergence, and the product acts as a germination booster, stimulating seed germination and root growth, besides increasing the plant nutrient uptake.

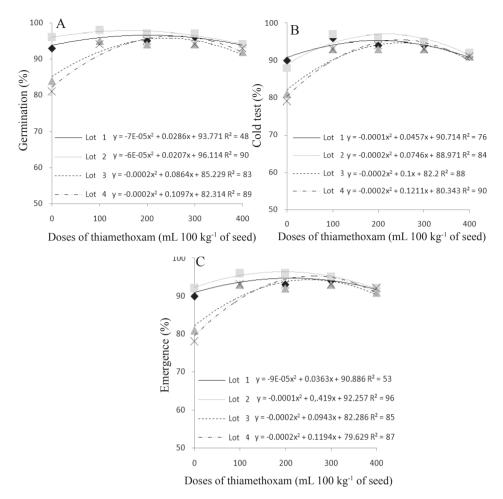


Figure 4. A - Germination (%)/Thiamethoxan doses (mL.100 kg<sup>-1</sup> of seeds); B - Cold Test (%)/Thiamethoxan doses (mL.100 kg<sup>-1</sup> of seeds); C - Emergence (%)/Thiamethoxan doses (mL.100 kg<sup>-1</sup> of seeds) of four lots of IRGA BR 424 cultivar rice seeds.

## Conclusions

The rice seed treatment with thiamethoxam positively favors the seed quality. Treatment doses between 100 and 200 mL per 100 kg of seeds were more effective to improve the physiological performance of rice seeds in most of the tests.

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