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Physiological potential of *Oryza sativa* seeds treated with growth regulators at low temperatures[1]

Potencial fisiológico de sementes de *Oryza sativa* tratadas com reguladores de crescimento em baixa temperatura

Mara Grohs[2]*, Enio Marchesan[2], Marcos Belinazzo Tomazetti[3], Rodrigo Roso[2] and Tiago Constante Formentini[3]

ABSTRACT - The rapid and uniform establishment of rice crops is important for improving production. However, this condition is influenced by several factors, including the soil temperature when planting, which may delay seed germination and compromise the final stand. The aim of this study was to evaluate the behaviour of substances which have the effect of growth regulator when applied to the seeds of different rice cultivars under low-temperature conditions. The experiment was carried out in a completely randomised design with four replications in a bi-factorial scheme, where factor A was represented by the different products (gibberellic acid - AG_3, tiamethoxam - TMX, Haf Plus® - HAF, and a control with water - TEST), and factor B by the irrigated rice cultivars (IRGA 424, IRGA 425, Puitá INTA CL, and Avaxi CL). In addition, the experiment was repeated at temperatures of 17 °C and 25 °C in order to simulate low-temperature conditions. The results showed that AG_3 is effective in increasing seed vigour in the rice cultivars at both temperatures, with the AG_3, TMX and HAF responsible for increasing germination percentage only at the temperature of 17 °C. The effect of the products is more pronounced at low temperatures, and is dependent on the cultivar; in cultivars which are sensitive to cold there is no effect from the products used.

Key words: Irrigated Rice. Phytohormone. Insecticide. Organo-mineral fertilizer.

RESUMO - O rápido e uniforme estabelecimento de uma lavoura de arroz é importante para aprimorar a produção. No entanto, essa condição sofre influência de diversos fatores, entre eles a temperatura do solo no momento da semeadura, a qual poderá atrasar a germinação das sementes e comprometer o estande final. O objetivo do presente trabalho foi avaliar o comportamento de substâncias com efeito de regulador de crescimento aplicados em sementes de diferentes cultivares de arroz irrigado em condições de baixa temperatura. Para tanto, o experimento foi desenvolvido no delineamento inteiramente casualizado com quatro repetições em esquema bifatorial, onde o fator A foi representado pelos diferentes produtos (ácido giberélico - AG_3, tiametoxam - TMX, Haf Plus® - HAF e testemunha com água - TEST); e o fator B, pelas cultivares de arroz irrigado (IRGA 424, IRGA 425, Puitá INTA CL e Avaxi CL). Além disso, o experimento foi reproduzido sob as temperaturas de 17 °C e 25 °C a fim de simular condições de baixas temperaturas. Os resultados indicaram que AG_3 é eficiente em aumentar o vigor das sementes das cultivares de arroz nas duas temperaturas, sendo AG_3, TMX e HAF responsáveis por aumentar a porcentagem de germinação final apenas à temperatura de 17 ºC. O efeito dos produtos é mais pronunciado em baixa temperatura e é dependente da cultivar utilizada, sendo que em cultivares sensíveis ao frio não há influência dos produtos utilizados.

Palavras-chave: Arroz irrigado. Fitohormônio. Inseticida. Fertilizante organo-mineral.

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INTRODUCTION

The reproductive stage of irrigated rice occurring during the period of greatest solar radiation is fundamental to obtaining high yields. This can be achieved by choosing the appropriate period for sowing, which must be carried out in order to ensure that the thermal load is sufficient to reach the reproductive stage during a period of favourable climatic conditions, i.e. a low probability of cold, and maximum incidence of solar radiation (CRUZ, 2010). However, with certain cultivars, this period may occur during the months of the year when the soil temperature is below the ideal for seed germination, offering adverse conditions and greater risks to the initial development of the plants.

The germination process includes a sequence of strongly temperature-dependent reactions, such as the breakdown of reserve substances, and transport and resynthesis in the embryonic axis (MARCOS FILHO, 2005). The cold is considered one of the main abiotic stresses in rice (YADAV, 2010), determining changes in growth patterns and seedling development, mainly due to affecting the expression of isoenzymes involved in the germination process (MERTZ *et al*., 2009). At the beginning of the sowing season, the soil temperature is often below 20 °C in the main rice-growing regions of the state of Rio Grande do Sul (STEINMETZ *et al*., 2008); the lower the temperature, the longer the germination process, reaching a point where metabolism is inactivated and the seed does not germinate (YOSHIDA, 1981). For the same author, establishment of the crop and the physiological potential of the seeds in the field are very dependent on the conditions of soil and climate under which the seeds develop. In addition, it is necessary to consider varietal differences in germination under different temperature conditions, and determine the different responses, which depend on the cultivar used.

There are currently substances that act on the metabolic pathways of plants, activating enzymes, increasing the rate of imbibition that affects the germination process, and determining greater vigour, stature, rooting, and nutrient absorption, among other benefits. These substances have the effect of growth regulators, with which previously treated seeds can become more tolerant to stress factors (ALMEIDA *et al*., 2011; LAUXEN; VILARELA, SOARES, 2010) and develop under suboptimal growing conditions with a greater chance of achieving high yields. Among these substances, gibberellin, a plant hormone considered a regulator of seed germination, is an important compound, due to inducing the production of enzymes such as α-amylase, which is responsible for starch degradation in the endosperm, a source of energy in germination (TAIZ; ZEIGER, 2013). In an experiment conducted by Bethke *et al*. (2006), a half of rice grains with no embryo showed activation of α-amylase-encoding genes after treatment with gibberellic acid (AG_3). In addition to AG_3, organo-mineral fertilisers such as Haf Plus®, obtained from natural extracts and algae, can have varied hormonal and nutritional effects on the plant. Furthermore, insecticides, whose benefit is usually evaluated for pest control, may have different effects on plant physiology; as an example, Almeida *et al*. (2011) found improvements in the physiological quality of rice cultivars treated with thiamethoxam (3-[2-chloro-thiazol-5-ylmethyl] -5-methyl- (1,3,5) oxadiazinan-4-ylidene-N-nitroamine), an insecticide of the neonicotinoid family.

The aim of the present study was to evaluate the behaviour of Haf Plus®, thiamethoxan and gibberellic acid on physiological potential in seeds of the irrigated rice cultivars IRGA 424, IRGA 425, Puitá INTA CL and Avaxi CL, sown at optimal (25 °C) and suboptimal (17 °C) temperatures.

MATERIAL AND METHODS

The experiment was carried at the Federal University of Santa Maria, in the state of Rio Grande do Sul (RS), using a biochemical oxygen demand (BOD) incubation chamber with a temperature accuracy of ± 0.3 ºC.

The experimental design was a bi-factorial scheme with four replications (4x4), factor A comprising four cultivars of irrigated rice (Puitá INTA CL, IRGA 424, IRGA 425 and Avaxi CL), and factor B comprising treatment of the respective seeds with gibberellic acid (AG_3), thiamethoxam (TMX), Haf Plus® (HAF) and the control with water. HAF is composed of nitrogen (5%) + organic matter (25%), L-α free amino acids (6%), extract of Ascophyllum nodosum algae, polysaccharides and micronutrients (0.72%). The doses used for 100 kg of seeds were 0.004 kg, 0.07 kg and 0.2 L of AG_3, TMX and HAF respectively. In order to simulate conditions similar to those found by the seeds when sown at the beginning of the recommended period, the experiment was carried out at temperatures of 17 °C (below the optimal limit for plant germination and establishment) and 25 ºC (within the optimal limit for these stages (STEINMETZ *et al*., 2008).

Treatment with the products was carried out directly on the seeds 24 hours before sowing, using a pressurised valve at a flow rate of 4.6 L 100 kg^{-1} of seeds. Methylene blue was added to the solution so that uniformity of the spray could be checked.

The variables to be analysed were germination percentage by the germination test, shoot and root length, and germination percentage of the lower quality seeds, obtained by artificial ageing.

For the germination test, the seeds were placed on 'germitest' filter paper, moistened with an amount of distilled water equal to 2.5 times the dry weight of the paper. Germination was recorded every 96 hours for 14 days, the seeds being considered as germinated when the root and coleoptile reached a length of two centimetres, as specified in the Rules for Seed Analysis (BRASIL, 2009). For both temperatures, the evaluations began six days after sowing (6 DAS), with the results expressed as a percentage of normal seedlings. To evaluate root and shoot length in the seedlings, four replications of 10 seeds were placed on a line drawn on the upper third of the paper. The seeds were kept for 14 days in a BOD chamber and the shoot and root length of normal seedlings was evaluated every 96 hours, using a ruler graduated in millimetres. Measurement began when 50% of the seeds had germinated; for the temperature of 17 °C, evaluation began at 10 DAS, and at 25 °C, evaluation started at 6 DAS. Values were taken as the sum of the measurements for each replication divided by the number of seedlings.

Complementary to the above, the effect of the products on aged seeds was evaluated through the accelerated ageing test (ELLIOT, 1982). The seeds were placed in a single layer on a screen suspended inside 'gerbox' plastic boxes. A volume of 0.04 L of distilled water was added to the bottom of the 'gerbox', and the boxes were covered and kept at 42 °C for 96 hours. After the ageing period, the seeds were treated with the products, and submitted to the germination test employing the same methodology as described previously, with evaluations at 7 and 14 DAS (BRASIL, 2009).

Statistical analysis was carried out by Scott-Knott test at 5% probability, with the germination percentage transformed by equation 1.

$$1: yt = \sqrt{y} + 1 \qquad (1)$$

RESULTS AND DISCUSSION

Gibberellic acid (AG_3) produced the greatest vigour irrespective of temperature, represented by the first count at six days after sowing (6 DAS), (Figure 1). After the first evaluation, the response to the use of the products became dependent on the cultivar at both temperatures. Stimulation of germination occurred in the treatment with AG_3 because hydrolytic enzymes involved in the process are synthesised more quickly in response to the presence of this compound, being transferred to the aleurone layer of the seed, where they promote conversion of the starch to sugar, used in the growth of the new seedling (LAUXEN; VILLELA; SOARES, 2010).

Seeds of the IRGA 424 and IRGA 425 cultivars, classified as sensitive to cold and of low initial vigour, did not at first (6 DAS) respond to any of the products used at 17 ºC. Seed treatment that promoted greater initial vigour in these cultivars would be a good management strategy, since genotypes of the Indica subspecies display a lower tolerance to cold (CRUZ; MILACH, 2004; MERTZ *et al*., 2009), especially the IRGA 424 cultivar, which had a survival rate of only 2.5% of seedlings in the test for cold sensitivity (CRUZ; DUARTE; CABREIRA, 2010).

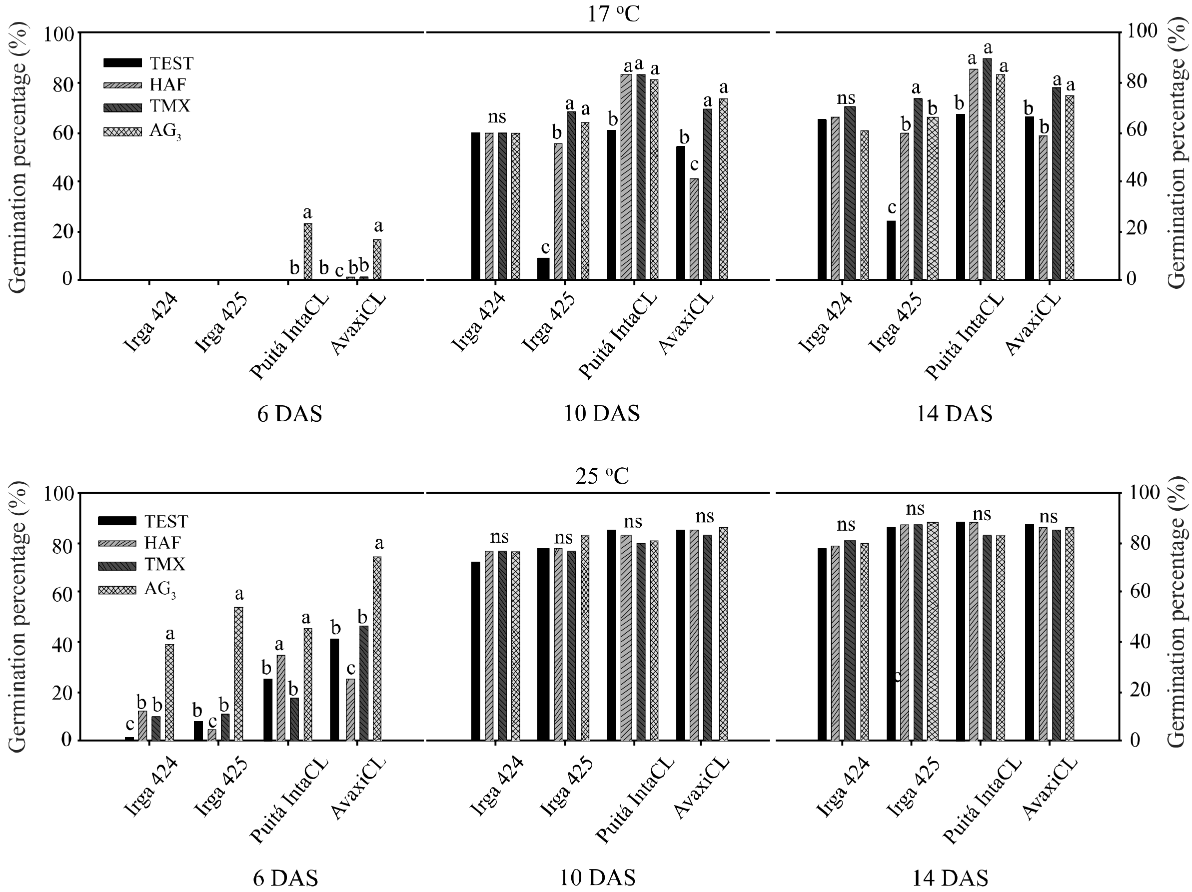
The evaluation made at 10 DAS showed a germination percentage of around 60% in the IRGA 424 cultivar, this being however the only cultivar that did not respond to the treatments at 17 °C. Seeds of the IRGA 425 cultivar had the lowest germination percentage of all the cultivars tested at 17 °C in the treatment with water (TEST); nonetheless, they responded most to the treatments, achieving increases in germination percentage in relation to the control of 36% for HAF, 42% for AG_3 and 50% for TMX. This result is important in the sense that IRGA 425 is a medium-cycle cultivar, requiring early sowing where the probability of low soil temperatures is greater.

It has already been verified that insecticides of the neonicotinoid family, such as thiamethoxam, can induce plant defences through stimulation of the production of salicylic acid, which is a phytohormone known for its role in defending the plant against pathogens, and as an inducer of acquired systemic resistance, also being able to regulate the response to such abiotic stresses as temperature, and to influence the antioxidant system of the plant (FORD *et al*., 2010; HORII; MCCUE; SHETTY, 2007). However, it is assumed that under adequate conditions of light, temperature and nutrients, the behaviour of thiamethoxam is stable, with no interference in germination (MACEDO; CASTRO, 2011).

Seeds of the Puitá INTA CL cultivar and the Avaxi CL hybrid displayed higher initial vigour and final germination percentage in the TEST treatment at the temperature of 17 °C in relation to the seeds of the other cultivars, except in the evaluation at 10 DAS, when the seeds of IRGA 424 surpassed those of Avaxi CL. When subjected to the treatments and temperature of 17 °C, there was an increase in percentage of germinated seeds in the Puitá INTA CL cultivar of 18, 22 and 16% for HAF, TMX and AG_3 respectively. The seeds of the Avaxi CL cultivar only responded to TMX and AG_3, showing an increase in germination percentage in relation to the control of 13% and 11% respectively.

From 10 DAS, and at the temperature of 25 °C, the differences between the products and the control became less pronounced or even non-existent. This demonstrates

Figure 1 - Germination percentage at 6, 10 and 14 days after sowing (DAS) in seeds of the IRGA 424, IRGA 425, Puitá INTA CL and Avaxi CL cultivars of *Oryza sativa*, treated with Haf Plus® (HAF), thiamethoxam (TMX)), Gibberellic acid (AG_3), and the control with water (TEST), at a temperature of 17 °C and 25 °C



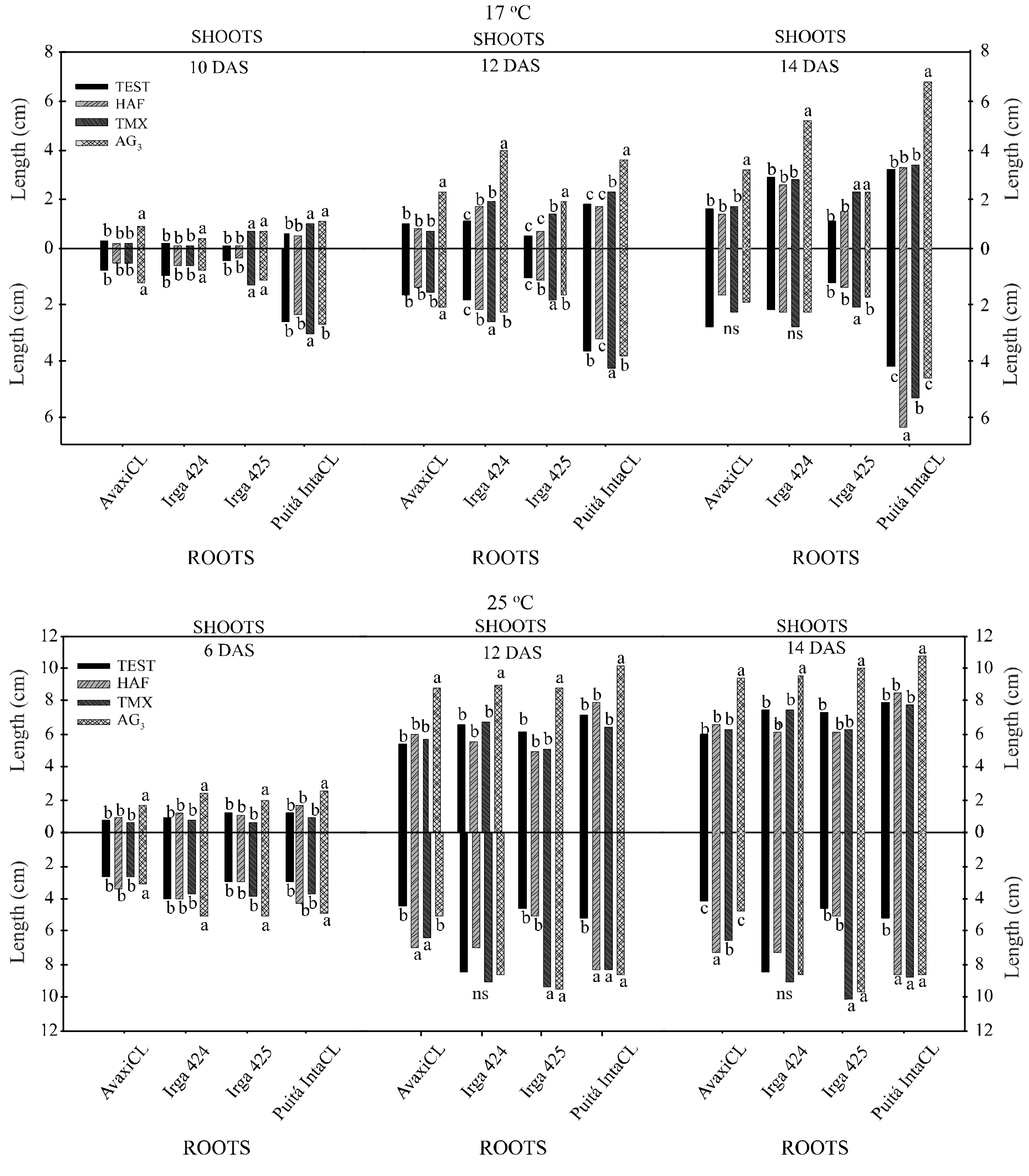
that the use of these products is effective only in those situations where germination is delayed, for example, by low temperatures at the time of sowing, and become less effective as stress conditions are minimised.

The post-germination stage was characterised by evaluating the shoot and root length (Figure 2). Plants of all the cultivars displayed an increase in shoot length when the seeds were treated with AG_3, at a temperature of both 17 °C and 25 °C.

This correlates with the fact that that the AG_3 may have acted mainly on the seedling shoots, since gibberellins promote elongation of the shoot by increasing the plasticity of the cell wall, followed by hydrolysis of the sugar, reducing water potential in the cell, and resulting in the entry of water and elongation of the seedling, especially in the mesocotyl region (LEE *et al*., 2012). In addition, gibberellin induces the transcription of genes associated with transitions that occur during the mitotic cycle, accelerating cell division (TAIZ; ZEIGER, 2013).

When TMX was used, there was better development of the root system in seedlings from most of the cultivars, especially at the temperature of 17 ºC. According to Cataneo *et al*. (2010), thiamethoxam accelerates the germination process by stimulating peroxidase activity, which can act both in the consumption of oxygen reactive species (EROs), preventing oxidative stress, and in the production of EROs, which would stimulate cell elongation, and which in this case, would occur in the radicular region. Stimulation of root development was more pronounced in plants of the IRGA 425 cultivar, reaching a difference of almost five centimetres with the use of TMX and AG3 in relation to TEST at 14 DAS and 25 °C. Considering the better adaptability of this cultivar to a system of pre-germination, and that mechanical stress, such as the removal of plants from the soil by the wind, are common under such a system, better development of the root system is desirable, reducing problems with securing the plants, and contributing to their initial establishment.

Figure 2 - Shoot and root length at 10, 12 and 14 days after sowing (DAS) at 17 °C, and 6, 10 and 14 DAS at 25 °C, in seeds of the IRGA 424, IRGA 425, Puitá INTA CL and Avaxi CL cultivars of *Oryza sativa*, treated with Haf Plus® (HAF), thiamethoxam (TMX)), Gibberellic acid (AG_3), and the control with water (TEST)

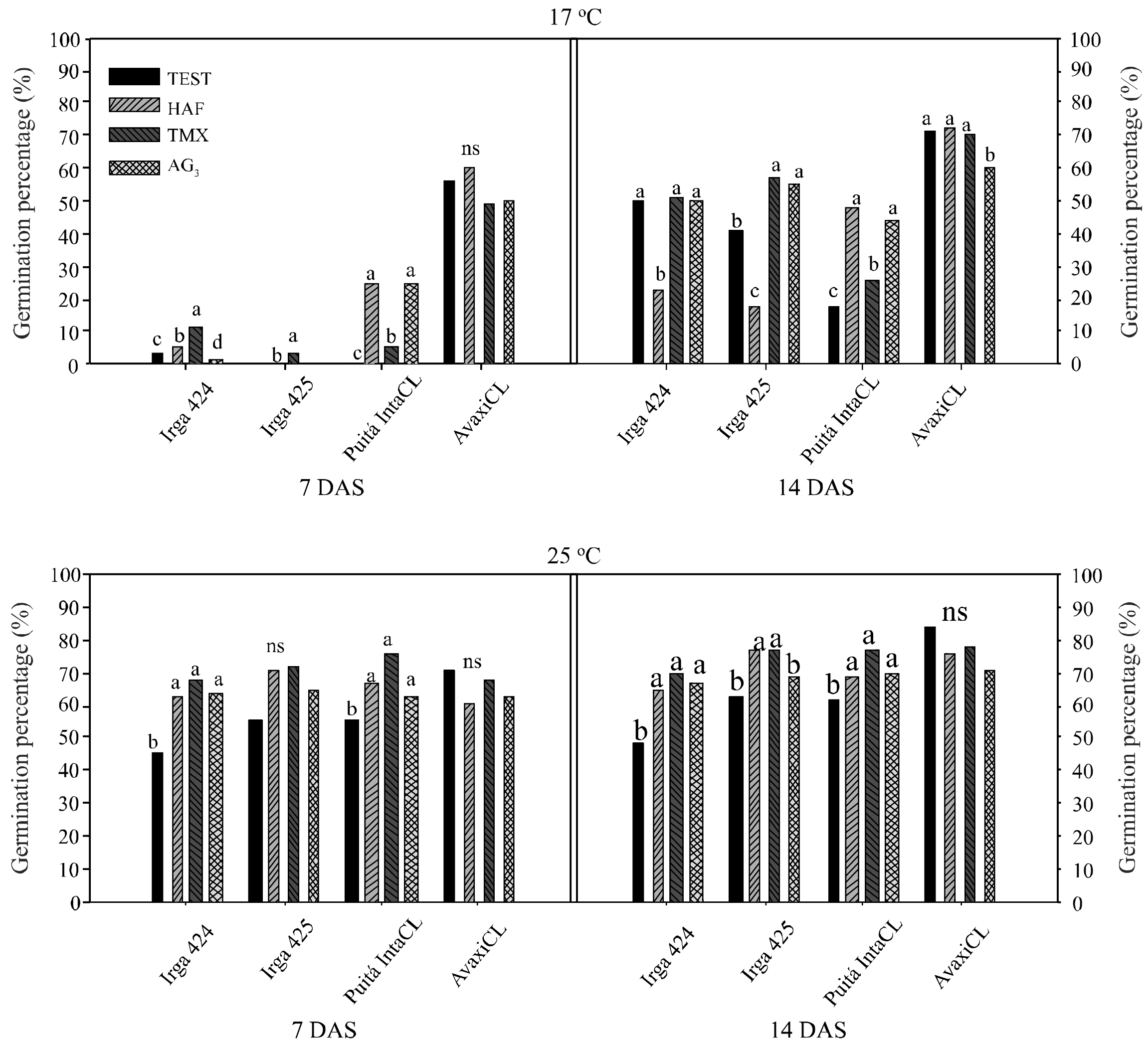


Treatment with HAF had a markedly smaller effect than TMX and AG_3 at the temperature of 17 °C, where in most cases, its effect was statistically equal to TEST. At the same temperature, HAF had a positive effect in various cases, depending on the cultivar used and the period of evaluation, but did not show any trend which would indicate benefits from its use. Similarly, plant development at 25 °C showed no positive effect from HAF, except on the root system of the Avaxi CL cultivar evaluated at 14 DAS. In some cases, such as IRGA 424 and IRGA 425, this product acted on both the root system and the shoots, producing more uniform plants, unlike AG_3 for example.

In the final part of the study, when evaluating the effect of the products on seeds of lower quality (Figure 3), it was found that response to the treatments with TMX and HAF was stronger when compared to AG_3, since the latter did not show any great difference in relation to the other products, and in some cases, such as the Avaxi CL cultivar, presented lower germination, even compared to the control. In addition, a large number of abnormal seedlings were seen in the treatment with AG_3 at the temperature of 17 °C, with excessive shoot development and no growth of the root system.

However, no references to such an effect on lower quality seeds were found in the literature. Forced ageing possibly acts on several aspects of the metabolism of these seeds which may be linked to the activation of and/or response to the use of AG_3, such as hydrolytic enzymes, some stage of cell division, or the gibberellin molecule, itself endogenous to the plant. By artificially ageing the seeds, it is possible to induce changes in the isoenzymatic profiles (acid phosphatase and peroxidase), resulting in a deteriorative effect caused by the exposure of the seeds to stress conditions, as well as reducing sugar content (KAPOOR *et al*., 2011). As the activity of gibberellins directly influences the performance of enzymes and the degradation of sugars (HUANG *et al*., 2015), this may inhibit response to the use of AG_3.

Figure 3 - Germination percentage in artificially aged seeds, recorded at 7 and 14 days after sowing (DAS) in seeds of the IRGA 424, IRGA 425, Puitá INTA CL and Avaxi CL cultivars of *Oryza sativa*, treated with Haf Plus® (HAF), thiamethoxam (TMX)), Gibberellic acid (AG_3), and the control with water (TEST), at 17 °C and 25 °C



CONCLUSIONS

1. Gibberellic acid increases seed vigour in cultivars of *Oryza sativa* studied at optimal (25 ºC) and suboptimal (17 ºC) growth temperatures, except in the IRGA 424 and IRGA 425 cultivars at the sub-optimal temperature;
2. Final germination percentage in the seeds at 17°C increases with the use of the tested products, except in the IRGA 424 cultivar. Considering the temperature of 25 °C, none of the products tested affects germination;
3. Gibberellic acid gives greater growth of the shoots, thiamethoxam of the root system, and Haf Plus® favours growth of the root system in the Avaxi CL cultivar at 25 °C;
4. The use of these products may be relevant during periods of early sowing in the soil, under low temperature conditions, in the state of Rio Grande do Sul.

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