RESEARCH NOTE

Treatment of rice seeds with salicylic acid: seed physiological quality and yield[1]

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ABSTRACT – Seed treatment with growth regulators, especially salicylic acid, is a promising alternative to the seed industry because it is an important inducer of resistance to diseases and pests, as well as acting significantly on quality and seed yield. The objective of this study was to evaluate the performance of rice seed treated with different concentrations of salicylic acid, as well as assess the crop yield and seed quality. The treatments consisted of increasing levels of 0, 50, 100, 150 and 200 mg.L^{-1} salicylic acid. To this was prepared a stock solution of salicylic acid and the highest concentration by successive dilution in distilled water, the other concentrations were obtained. The physiological quality of seeds produced was treated and evaluated by tests of vigor and germination, and after harvest were evaluated seed yield. It follows that treatment of rice seeds with salicylic acid concentrations up to 130 mg.L^{-1} at a dose of 2 mL.kg^{-1} seed does not affect the germination and affects the strength, however provides substantial increases in the yield of seeds. The seed treatment with salicylic acid has no influence on seed quality produced.

Index terms: *Oryza sativa* L., germination, vigor, growth regulator.

Tratamento de sementes de arroz com ácido salicílico: qualidade fisiológica e rendimento da cultura

RESUMO – O tratamento de sementes com reguladores de crescimento, principalmente o ácido salicílico, é uma alternativa promissora ao setor de sementes por se tratar de um importante indutor de resistência de doenças e pragas, além de atuar significativamente na qualidade e no rendimento de sementes. Objetivou-se com o presente trabalho avaliar o desempenho de sementes de arroz tratadas com diferentes concentrações de ácido salicílico, bem como avaliar o rendimento da cultura e a qualidade das sementes. Os tratamentos constaram de níveis crescentes de 0, 50, 100, 150 e 200 mg.L^{-1} de ácido salicílico. Para isso, preparou-se uma solução estoque de ácido salicílico na maior concentração e através de diluições sucessivas em água destilada, foram obtidas as demais concentrações. A qualidade fisiológica das sementes tratadas e produzidas foi avaliada por testes de vigor e de germinação, sendo que após a colheita avaliou-se o rendimento de sementes. Conclui-se que o tratamento de sementes de arroz com ácido salicílico até a concentração de 130 mg.L^{-1}, na dose de 2 mL.kg^{-1} de semente, não influencia a germinação ou afeta o vigor, entretanto proporciona incrementos substanciais no rendimento da cultura. O tratamento das sementes com ácido salicílico não apresenta influencia na qualidade das sementes produzidas.

Termos para indexação: *Oryza sativa* L., germinação, vigor, regulador de crescimento.

Introduction

Rice yield in Brazil, in the 2010/2011 harvest, was 12.6 million tons, and the state of Rio Grande do Sul produced 8.2 million tonnes, equivalent to approximately 65% of national production (CONAB, 2011). Rice is a major cereal consumed by the world population, representing about 30% of world production of grains (Yadav and Jindal, 2008; Luangmalawat et al., 2008).

Salicylic acid is a natural compound that plays a central role in certain physiological processes and defense responses

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in plants (Shi and Zhu, 2008). It can affect seed germination, cell growth, stomatal opening, expression of genes associated with senescence, and fruit production (Klessig et al.,, 2009). This effect was also observed by Guo et al. (2009) who have revealed that pretreatment of rice seeds with salicylic acid has inhibited the activity of catalase and increased the levels of hydrogen peroxide, increasing the resistance of plants treated with cadmium, thus relieving oxidative stress and increasing plant tolerance to cadmium. The application of exogenous salicylic acid has also increased resistance in plants. The effect of exogenous salicylic acid applied in infected tomato roots on damage resistance to the *Fusarium oxysporum f.* fungus has been assessed by Mandal et al. (2009) who found positive effects.

Salicylic acid is the first plant derivative phenolic compound to induce systemic acquired resistance (Araujo et al., 2005). It is involved in a variety of physiological processes, and is included in a new class of plant growth regulating substances. This compound is found in leaves, inflorescences of thermogenic plants, and in plants attacked by pathogens (Castro and Vieira, 2001). A detailed analysis on 34 species considered significant for agriculture, such as rice, soybean, and barley, has confirmed the distribution of salicylic acid at levels above 1µg.g^{-1} of fresh material such as in leaves and reproductive structures (Raskin et al., 1990).

Salicylic acid is a growth regulator synthesized from the phenylalanine amino acid, a biosynthesis intermediate of most phenolic compounds. There are two basic metabolic pathways that are involved in the synthesis of phenolic compounds: the shikimic acid pathway and the malonic acid pathway. The first one participates in the biosynthesis of most plant phenolics (Taiz and Zeiger, 2004). Such pathway generates aromatic amino acids such as phenylalanine, which through the elimination of a molecule of ammonia, by the action of phenylalanine amonialiase, forms cinnamic acid, which can, in turn, be converted to ortho-coumaric acid or benzoic acid that can form salicylic acid (Arteca, 1995).

Salicylic acid has many roles, mainly to inhibit germination and growth; to interfere in root absorbance, reduce transpiration, and cause leaf abscission (Kerbauy, 2008). However, according to Carvalho et al. (2007), the application of salicylic acid in marigold seeds, medicinal plant belonging to the *Asteraceae* family, has positively contributed to germination and to the rate of germination speed, whereas levels of water stress and heat to 35 °C. Maia et al. (2000) have found that salicylic acid promoted increased germination percentage in soybean seedlings, besides stimulating the length of roots and increasing green matter. Salicylic acid has shown the ability to activate peroxidase having an important role in the biochemical process, such as the biosynthesis of lignin and suberin that are involved in cell stress, and these substances are of great importance for the protection of plants (Sakhabutdinova et al., 2004).

Some growth regulators, called elicitors, favor the efficiency of metabolic processes, or act directly on metabolic pathways in response to unfavorable environments, allowing for adaptations to environmental changes; and one of these substances is the salicylic acid. The study of these substances, and of possible cross-responses, is important for agriculture, especially in view of the future prospects of environmental changes such as the greenhouse effect. Moreover, the emergence and early seedling establishment in the field are considered the most critical phases of crops, due to sensitivity to adverse abiotic factors. Thus, studies that seek this information, besides aiming to learn which products can be used to stimulate seed yield and physiological quality, should be encouraged.

Within this context, the aim of this present study was to assess the performance of rice seed treated with different concentrations of salicylic acid, as well as assess seed yield and quality.

Material and Methods

This work was conducted in the Laboratory of Seed Analysis and in greenhouse, at the Eliseu Maciel School of Agronomy from the University of Pelotas. BRS Querencia cultivar rice seeds were used.

Treatments consisted of increasing levels of 0, 50, 100, 150 and 200 mg.L^{-1} of salicylic acid, and for that, we prepared a stock solution of salicylic acid ($C_7H_6O_3$; 138.12 g.mol^{-1}) in the greatest concentration (200 mg.L^{-1}) and through successive dilutions in distilled water, the other concentrations were obtained. The seeds were treated and applied to 2 mL per kg of seed in each respective concentration of salicylic acid, according to methodology described by Nunes (2005), and the manual method was adopted using polyethylene bags. The solution of salicylic acid with the different doses was directly placed at the bottom of the plastic bag to a height of approximately 0.10 meters. Then, we placed 0.200 kg of seeds within the bag. They were agitated for 3 minutes. Right after, seeds were left to dry at room temperature for 24 hours. The spray volume was 6 mL per kg of seed, with the addition of 4 mL of distilled water.

The physiological quality of the treated seeds was assessed using the following tests: *Germination* (G) - conducted with four replicates of 50 seeds for each treatment, in germination paper substrate ("germitest") previously soaked in distilled water, using the 2.5 times the mass of dry paper proportion, and kept at 25 °C. Assessments were performed according to Rules for Seed Testing (Brasil, 2009), fourteen days after sowing. *First count of germination* (FCG) - consisted of determining the percentage of normal seedlings seven days after sowing, at completion of the germination test. *Accelerated aging* (AA) - horizontal gerbox with wire mesh fixed to middle position was used. We added 40 mL of distilled water to the bottom of each gerbox and seeds were distributed over the screen for each treatment, in order to cover the screen surface, constituting a single layer. Then the boxes containing seeds were capped and placed in the BOD incubator at 41 °C for 96 hours. After this period, the seeds were subjected to germination test as described above. Assessment took place after seven days, and results were expressed as a percentage of normal seedlings. *Cold test* (CT): we used four replicates of 50 seeds, distributed in germination paper substrate "germitest", previously moistened with distilled water, using 2.5 times dry paper weight. The rolls were placed inside plastic bags and kept in a refrigerator at 10 °C for seven days. After this period, the germination test was performed as described above. Assessment took place after 7 days and results were expressed as a percentage of normal seedlings. *Field emergence* (FE): held in beds containing soil, where seeding was performed manually at a depth of 2-3 cm, with four replications of 50 seeds for each treatment. Seedling emergence count was made 21 days after sowing. *Length of shoot and root* (LS and LR): was performed with four replicates of 20 seeds for each treatment. Substrate used was towell paper roll "germitest", in which the seeds were distributed in two longitudinal straight lines and staggered in the upper third of the paper. After making the rolls, they were placed in a germination chamber set at a constant temperature of 25 ºC (Nakagawa, 1999). On the seventh day after sowing, the total length of seedling, shoot and root of normal seedlings were measured and then the mean length of shoot and root was determined.

After the analysis of the physiological quality of treated seeds, 10 seeds were sown per pot with capacity of 15 dm^3 of soil, and at 5 days after seedling emergence (emergence considered with over 51% of emerged seedlings), we conducted the thinning of smaller seedlings, with 4 plants remaining per pot (larger) filled with sieved soil, collected from the A1 horizon of a solodic eutrophic Haplic Planossolo (Streck et al., 2008), pertaining to the Pelotas mapping unit. Fertilizers were applied according to soil test results and recommendations of the Commission of Soil Chemistry and Fertility - RS / SC, 2004. Only nitrogen, phosphorus and potassium were used, applied 14 days before sowing, and liming was performed thirty days before sowing. After sowing, the experimental units were manually watered daily, keeping soil near field capacity until water entry. The establishment of water blade occurred in early tillering, also with manual irrigation, where a height of 10 cm was maintained in the bucket until the final stage of the experiment.

Manual harvesting was conducted when plants were at stages R8 and R9, characterizing the physiological maturity of seeds. The physiological quality of seed was assessed with the following tests: *first count of germination (FCG), germination (G), accelerated aging (AA), cold test (CT)*, described above. *Seed yield* (Y): was determined by weighting harvested seed, where moisture was corrected to 13% and results expressed in gram.plant^{-1}.

The experimental design was randomized for both locations, greenhouse and laboratory tests. Data were subjected to analysis of variance and polynomial regression. Analyses were performed using the statistical program Winstat 2.0 (Machado and Conceição, 2003).

Results and Discussion

Figure 1 data show variations in rice treated seeds in different concentrations of salicylic acid via rice seed treatment. The variable *first count of germination (FCG)* adjusted to a linear model, while *cold test (CT)* and *field emergence (FE)* showed quadratic behavior. Rice seed treatment with salicylic acid did not affect the physiological quality of seed produced. Thus, data have not been discussed nor analyzed.

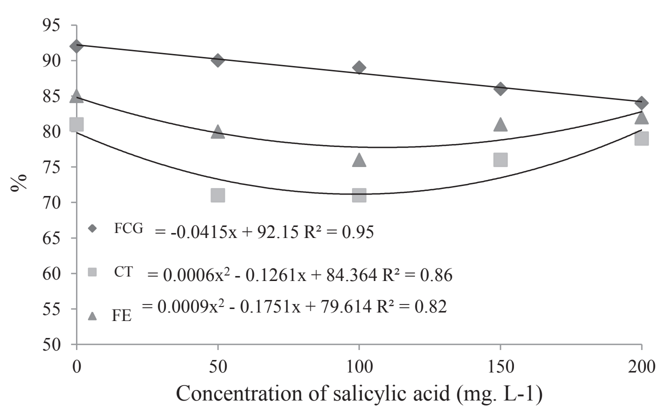

Figure 1. First count of germination (FCG), Field Emergence (FE) and cold test (CT) of rice seed treated with different concentrations of salicylic acid.

Regarding the vigor of rice seeds treated with salicylic acid, it was found decreased FCG and CT, with increasing concentration in the solution of up to 200 mg. L^{-1}. Results found disagree with Maia et al. (2000) who have assessed the effects of salicylic acid on soybean germination and vigor, concluding that it had a negative effect on germination, although it stimulates the activity of **α**-amylase. When using 0.5 mM salicylic acid, Szepesi et al. (2005) found increased percentage of germination in tomato seeds. With respect to seedling *field emergence* (FE) and in the cold test, we may note that rice seed treatment with different salicylic acid concentrations has affected seedling emergence, just as in the cold test.

The following results were indicated by the equations of each variable, where the values that best represent the application of salicylic acid to seeds were chosen. Given that, we found that up to a concentration of 97 $mg.L^{-1}$ there was significant reduction in FE, and up to 105 $mg.L^{-1}$ there was decreased CT. However, when concentration of salicylic acid was increased to 200 $mg.L^{-1}$, there was a significant increase in the number of seedlings and in the number of normal seedlings in CT, but these results were lower than zero. Maia et al. (2000) have observed an increase in the number of emerged seedlings in seeds treated with salicylic acid, and the most relevant contrasts were in relation to the zero dose (69%) and the 50 mg/kg dose (78%), and between the 50 and 100 mg/kg doses (67%).

Regarding seed germination, we did not find significant influence, however according to Silveira et al. (2000), salicylic acid applied to seeds of cv. EMBRAPA 7 TAIM showed inhibitory effect on germination. These results corroborate those of Kerbauy (2008) who considers salicylic acid a phenolic compound with different functions, mainly the inhibition of germination.

Figure 2 shows the trend line of shoot length (SL) of rice seedlings from treatment with different concentrations of salicylic acid. There is a reduction in the SL of seedling, and the concentration of 130 $mg.L^{-1}$ is the most significant one. Data disagree with those found by Maia et al. (2000), who have found in soybean that salicylic acid used alone has increased the lengths of shoots and roots and fresh weight at concentrations of 50 and 100 $mg.kg^{-1}$. The results of this study agree with those found by Kerbauy (2008), where salicylic acid tended to inhibit plant growth.

Figure 3 shows the positive effect of salicylic acid concentrations via seed treatment up to a concentration of 129 $mg.L^{-1}$ on yield, adjusted to a quadratic equation. The most effective concentration (129 $mg.L^{-1}$) produced approximately 18 $g.plant^{-1}$, with about 60% higher yield than concentration zero.

Treatment of rice seeds with salicylic acid, in general, did not impair the germination of treated seeds, nor produced seeds, and has positively affected seed yield up to a concentration of 130 $mg.L^{-1}$. However, it is important to note the need for more studies to examine the vigor of seeds treated with salicylic acid, and the initial growth of seedlings.

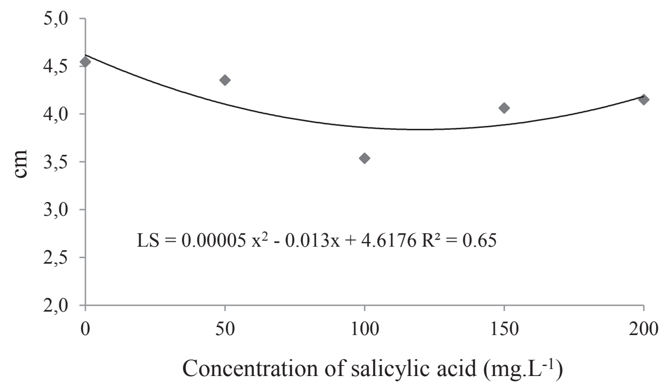

Figure 2. Length of shoot (LS) of rice seedlings from seed treatment with different concentrations of salicylic acid.

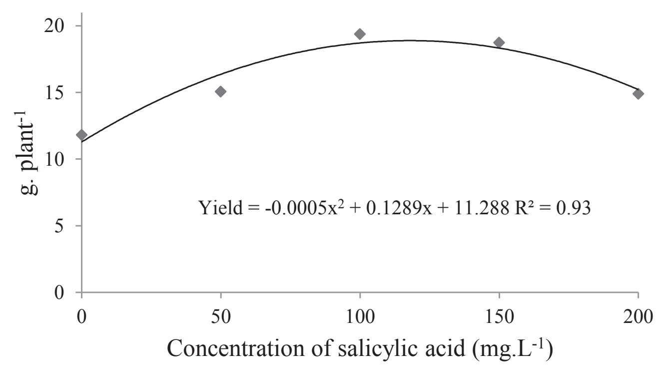

Figure 3. Rice seed yield from their treatment with different concentrations of salicylic acid.

Conclusion

Rice seed treatment with salicylic acid in concentration of 130 $mg.L^{-1}$at seed dose of 2 $mL.kg^{-1}$ did not affect germination, and it did affect seed vigor, however it provides substantial increases in seed yield. Seed treatment with salicylic acid has no influence on seed quality.

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