

Salicylic Acid Concentrations and Its Effects on the Physiological Quality of Soybean Seeds

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Abstract

The objective of this study was to evaluate the physiological quality of commercial soybean seeds submitted to different concentrations of salicylic acid (SA), directly on germination paper and gerboxes for 24 hours. Seeds of cultivars NA 5909 RG and Tec Irga 6070 RR were soaked in salicylic acid solutions with concentrations of zero, 250, 500, 750, 1000, 1500, 2000, 3000, 4000 and 5000 μM . Seed imbibition occurred in two ways: (1) germination paper moistened with salicylic acid solutions; (2) imbibition of the seeds in salicylic acid solutions in gerbox boxes for 24 hours and subsequent sowing on germinated paper moistened with distilled water. On the fifth day after sowing, the number of normal seedlings (first count), length, fresh and dry mass of root and shoot were determined. It was concluded that the concentrations of SA between 250 and 750 μM can be used in soybean seeds, however, above 1000 μM may impair the parameters of physiological quality. The gerbox method for 24 hours provided the best results without the drastic reduction of the parameters in the lowest concentrations of SA.

Keywords: vigor, germination, imbibition

1. Introduction

Soybean cultivation is of global importance in agribusiness because it is used for different objectives, both for animals and humans (Fioreze et al., 2011). The demand for higher productivity is achieved with the use of techniques and management, and the beginning of a crop will only have uniformity with the use of seeds with high physiological potential (Marcos Filho, 2015a). However, biotic and abiotic stresses may decrease yield throughout the growing season in the field, from germination to harvesting (Danquah et al., 2014; Jaleel et al., 2009; Morando et al., 2014).

To maintain its capacity to tolerate stresses, the plant uses its complex defense system, which is composed of different mechanisms and specific compounds that aid in this process (Yang & Dong, 2014). Among these, salicylic acid (SA) is considered a plant hormone present in numerous plant species and acts in important biochemical and physiological processes, such as growth and development, stomatal closure, nutrient absorption, chlorophyll and protein synthesis, foliar abscission and seed germination (Hayat, 2010; Miura & Tada, 2014; Vazirimehr & Rigi, 2014). In addition, it has an influence on the response to different types of stress, through chemical signaling in plant cells together with antioxidant complex enzymes (Janda et al., 2014; Parmoon et al., 2017). Also, other enzymes such as superoxide dismutase, peroxidases, catalases, among others, have the function of reducing the damage caused by free radicals, such as reactive oxygen species (ROS), which are formed mainly in moments of stress (Hayat et al., 2012; Lee, Kim, & Park, 2010).

Studies that perform the exogenous application of SA do not have consensus about the concentration to be used, the time of contact or route for absorption. There is concrete information that low concentrations of SA aid in antioxidant capacity; already, high concentrations may lead to oxidative stress or susceptibility to abiotic stress

(Miura & Tada, 2014). However, each situation may be influenced by the study conditions, which consequently increase the variability of the results. As an example, Kang et al. (2012) observed that high concentrations decreased important parameters in the wheat crop, such as lower seedling growth, increased activity of antioxidant enzymes, decreased photosynthesis, thus increasing plant stress levels.

Tang et al. (2017) point out that the concentration of 0.5 mM and osmotic potential of -1.03 MPa in the germination of soybean seeds reduced the effect of high water stress. Similarly, Al-Hakimi (2006) suggested that the concentration of 0.6 mM SA promoted a water deficit inhibitory effect on several parameters evaluated in leaves and roots. However, the role of SA and its various functions in the plant defense system is not yet known, so studies that specifically elucidate its performance are needed (Janda & Ruelland, 2015).

It is noticed that although there are several studies using SA, a pattern of concentrations and mode of imbibition of the compound after seed germination has not yet been established. Therefore, the objective of the study was to verify the effect of different concentrations of SA on some parameters of physiological quality in soybean commercial seeds.

2. Material and Methods

The study was conducted at the Laboratory of Seed Research and Development (LDPS) of the Department of Plant Science at the Federal University of Santa Maria, in the Rio Grande do Sul state. Commercial soybean seeds of Nidera® NA 5909 RG and Bayer®/Tec Irga 6070 RR cultivars produced in the 2015/2016 crop were used, both of which are recommended for cultivation in the state.

Initial evaluation of seeds: samples from both cultivars were evaluated separately for physical and physiological characteristics by performing the following tests: thousand seed weight, moisture content, first count of germination, germination test, root and shoot length, dry mass of seedlings, mass electrical conductivity and emergence of seedlings on field (Krzyzanowski; Vieira, & França Neto, 1999; Nakagawa, 1999; Brasil, 2009).

Physiological quality of soybean seeds submitted to SA concentrations: after the initial characterization, the seeds were submitted to germination under different concentrations of SA (Sigma-Aldrich®): zero; 250; 500; 750; 1000; 1500; 2000; 3000; 4000; 5000 μM . In the literature, there are different studies including soybean, but there is no consensus among researchers of the most adequate concentration (Al-Hakimi, 2006; Tang et al., 2017).

The SA was supplied to the seeds in two forms: (1) in the germinated paper moistened with the SA solutions; (2) imbibition of the seeds in SA solutions in gerbox for 24 hours. For the first group, four replications of 50 seeds were sown on rolls of germination paper with the SA solutions mentioned, in the proportion of 2.5 times the weight of the dry paper. The rolls were packed in plastic bags and kept in a BOD (Box Organism Development) germination chamber under constant light and temperature of 25 °C.

In the second option, four replicates of 50 seeds were placed in gerbox boxes to soak on three sheets of germinating paper moistened with 25 mL of SA solution at the same concentrations. The seeds remained soaked for 24 hours in BOD under constant light and temperature of 25 °C. After this imbibition period, the seeds were placed to germinate on germinated paper moistened only with distilled water, in the proportion of 2.5 times the dry paper mass. The rolls were packed in plastic bags, kept in BOD, under continuous light and at 25 °C (Brasil, 2009).

For both ways of SA treatment, on the fifth day after sowing, the percentage of normal seedlings was evaluated and, at the eighth day, the percentage of germination was estimated according to Brazil (2009). In addition to the germination test, on the fifth day after sowing, ten normal seedlings were removed in sequence from the upper part of the germinating roller, to compose the length, fresh and dry mass of root and shoot, being the cotyledons removed (Nakagawa, 1999).

Statistical analysis: the study was conducted in a completely randomized design with four replicates per treatment, each replicate being composed of 50 seeds. Initially, the assumptions of the mathematical model were verified by Action® software (Equipe Estatcamp, 2014). In case of non-compliance with the normality of the errors and homogeneity of the variances, the data were transformed by the methodology \sqrt{x} . The percent data were transformed by the equation: $\arcsin\sqrt{\%/100}$.

Seeds imbibition forms with SA (germination paper and gerbox for 24 hours) and cultivars were analyzed separately. Data were submitted to analysis of variance by the F test ($p < 0.05$) and regression analysis ($p < 0.05$) using the Sisvar® software (Ferreira, 2011).

3. Results and Discussion

The physical and physiological characterization of the seeds of the two commercial cultivars is presented in Table 1. It is observed that both have adequate humidity and germination above 80%, however it is noticed a difference in the physiological quality between these materials. The data of the initial characterization were not submitted to statistical analysis, once the objective was only to characterize the cultivars regarding the physiological quality. According to Marcos Filho (2015b), the correct characterization of seed lot quality is extremely important in the use of vigor tests and to understand the results that can be found in the most diverse studies.

Table 1. Thousand seed weight (TSW), moisture (M), first count (FC), germination (G), radicle length (RL), shoot length (SL), radicle dry mass (RDM), shoot dry mass (SDM), electrical conductivity (EC), field emergence (FE) and emergence speed index (ESI) of soybean seeds Tec Iriga 6070 RR and NA 5909 RG

Cultivar	TSW	Moisture	FC	G	RL	SL	RDM	SDM	EC	FE	ESI
	g	----- %	-----	-----	----- cm	-----	----- mg	-----	$\mu\text{S cm}^{-1} \text{g}^{-1}$	%	
Tec Iriga 6070 RR	137	12,97	83	92	7,6	14,9	87	198	55,8	97	14,3
NA 5909 RG	163	9,85	67	93	9,0	4,4	74,5	166,2	70,9	84	7,5

The data on vigor and germination for both cultivars and imbibition forms are shown in Figure 1. There was no significant difference in the germination for the cultivar Tec Iriga 6070 RR (Figures 1A and 1B). In general, it is noted that the vigor and germination are very similar for the two materials and imbibition method. Thus, at concentrations ranging from 250 and 1000 μM occurred the increase of the percentage values, however, from 1000 to 5000 μM these values decreased. In comparison with the control, concentrations of 500 and 750 μM demonstrated the highest values (Figures 1A-1D). Once there was no type of stress involved, these results were due to the concentrations of SA used.

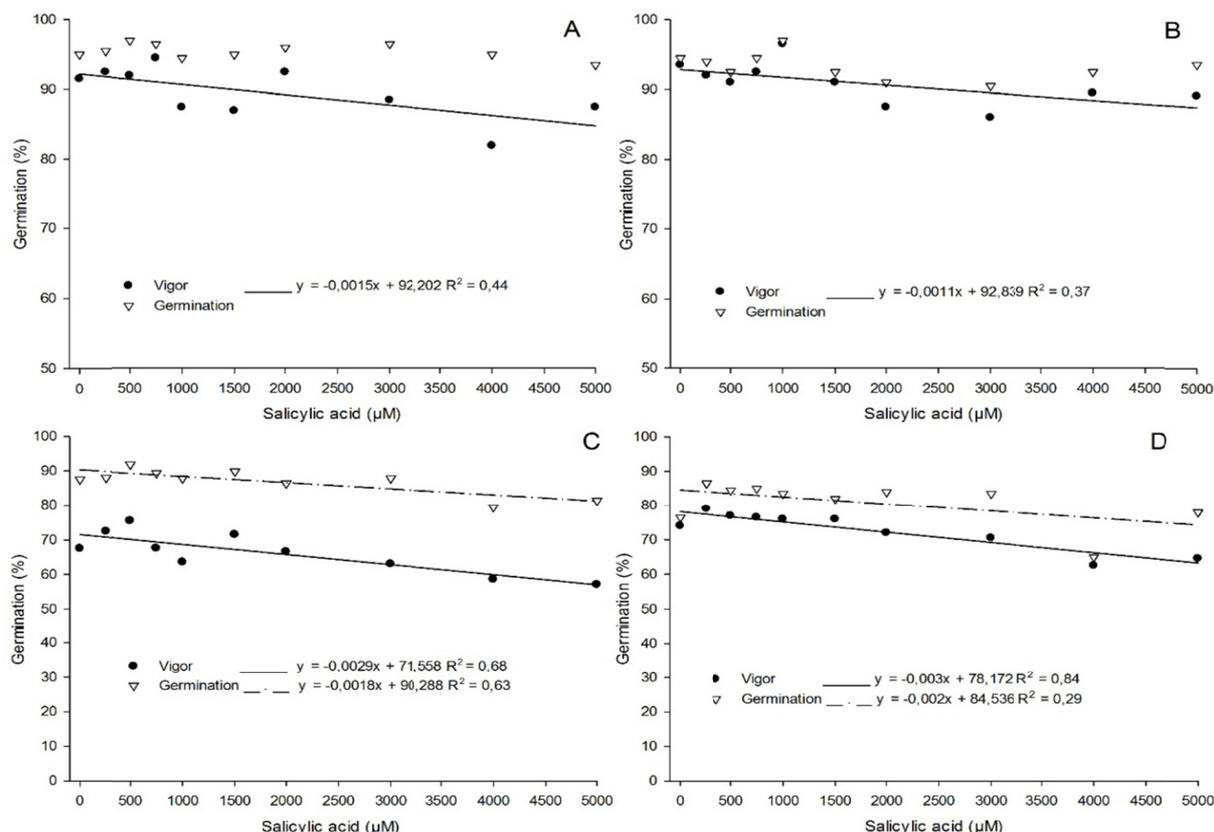


Figure 1. Percentage of vigor and germination for the plants Tec Iriga 6070 RR (A) paper, (B) gerbox and NA 5909 RG (C) paper and (D) gerbox

Most studies report that positive results with SA are usually found in the presence of stress. For example, Al-Hakimi (2006) detected increases in the cell wall metabolism of leaves and roots of soybeans using the concentration of 600 μM with the presence of water deficit. In the same context, Lee, Kim, and Park (2010) showed that under normal conditions during the germination process the SA is not essential, only in the stress situation.

Therefore, the significance of high and low concentrations of SA may vary according to species, type of stress evaluated, the period of imbibition or contact of seeds, absorption medium and even the parameters and target of study. Parmoon et al. (2017) evaluated the treatment of seeds of *Sylibum marianum* with SA, and concluded that concentration of 1000 mg L^{-1} considerably improved germination and vigor of the aged seeds and seedling growth, in addition to the greater antioxidant activity.

Interestingly, Siddiqui et al. (2018) used 100 μM of SA in the treatment of wheat seeds, and there was an increase in germination and plant height due to the increase of proline and total soluble carbohydrates that suppressed the formation of EROs in the roots. It is observed that this concentration is lower than that used in our study, therefore, depending on the species, the sensitivity and response to SA may be completely different.

Figure 2 shows the seedling lengths for each organ and total. Again, the behavior of both materials was similar, but on the germination paper, the values were lower for the three variables studied (Figures 2A and 2C). In gerbox there was an increase comparing the imbibition methods (Figures 2B and 2D). It is noteworthy that there was a different behavior for Tec Irga 6070 RR in the gerbox, which at the concentration of 5000 μM there was an increase in the values, the same did not occur in the paper and for the cultivar NA 5909 RG.

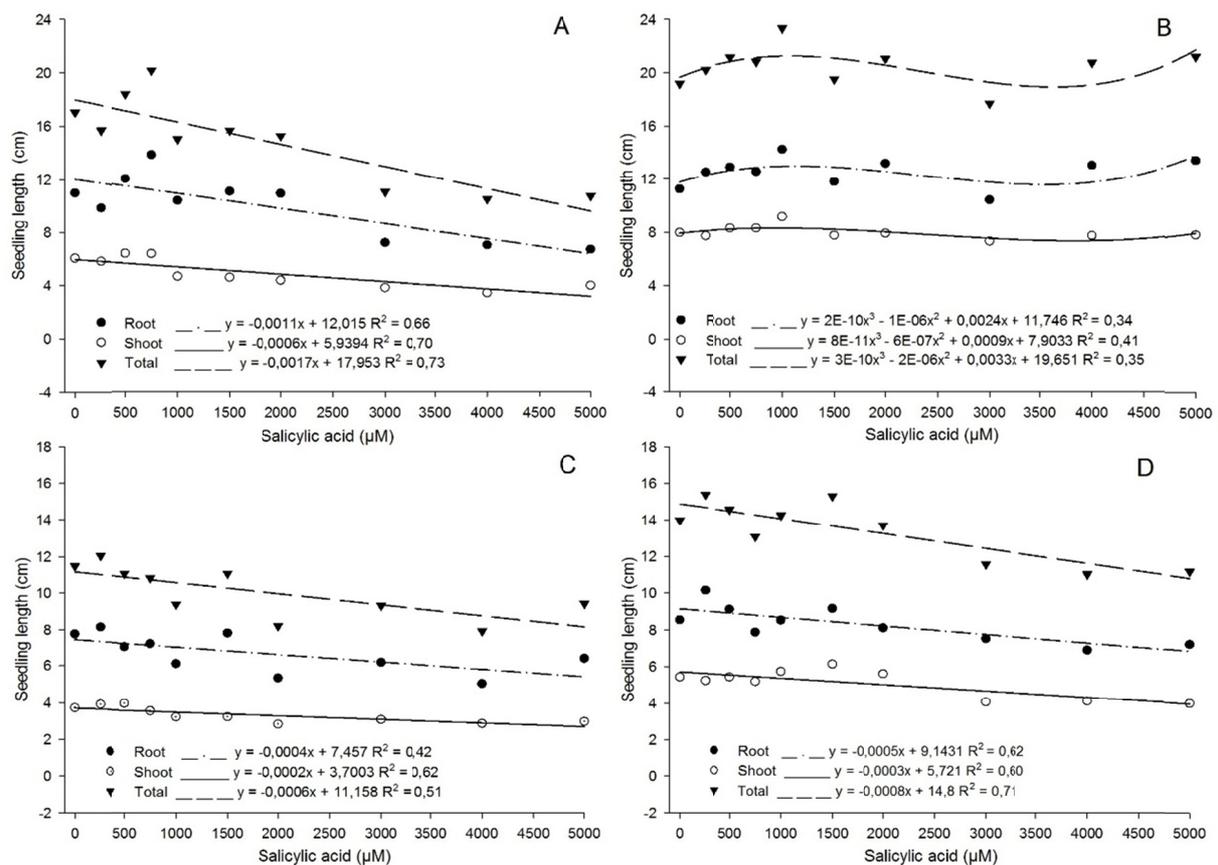


Figure 2. Root, shoot and total length for the cultivars Tec Irga 6070 RR (A) paper, (B) gerbox and NA 5909 RG (C) paper and (D) gerbox

The time of contact of the concentrations with the seeds in each method was different, and this factor may have contributed to the difference in SA absorption. Taking this into account, it is inferred that as a defense practice, at the highest concentrations, such as 4000 and 5000 μM , the seedling in an attempt to alleviate the possible stress caused, emitted root and shoot to overcome this moment, however, for NA 5909 RG this behavior was different.

High concentrations of SA lead to oxidative stress and an increase in EROs that impair cellular function at low concentrations, such as 10 μM SA, used by Lan et al. (2016) resulted in less accumulation of aluminum in soybean roots and in the activation of antioxidant enzymes, which helps to reduce the stress caused by this element.

The fresh mass values for the two cultivars and imbibition methods are described in Figure 3. Similar to that exposed for the dry mass variable (Figure 2), the fresh mass behavior exhibited a trend of higher weights for the gerbox method and smaller in the paper. In addition, the range of concentrations between 250 and 1000 μM , but mainly at 500 and 750 μM , showed the highest fresh root weight, shoot and total weight for both cultivars. Likewise, in the majority of cases, concentrations above 1000 μM affected the parameters evaluated (Figure 3).

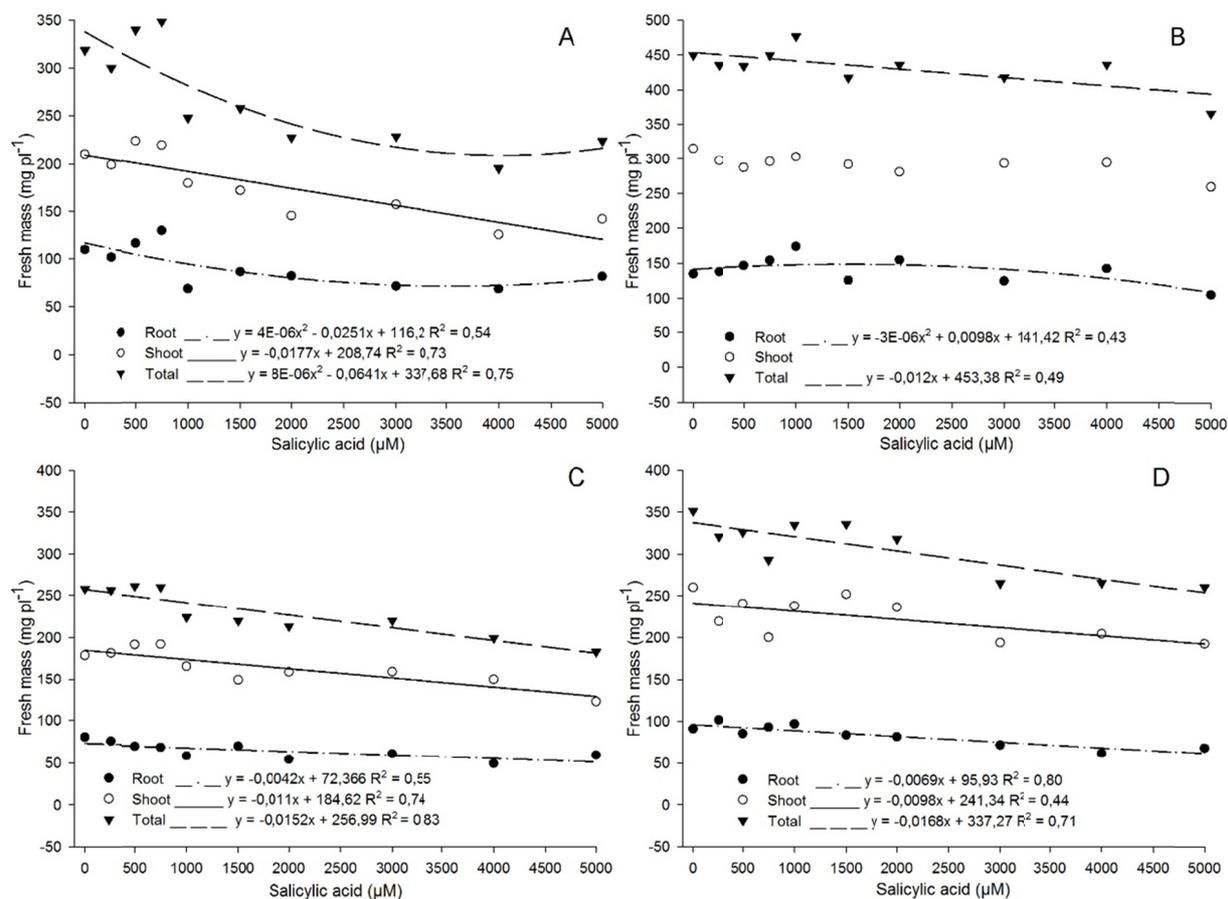


Figure 3. Fresh root, shoot and total mass for the cultivars Tec Iriga 6070 RR (A) paper, (B) gerbox, and NA 5909 RG (C) paper and (D) gerbox

These results are similar to those obtained by Coronado, López, and Saavedra (1998), where the foliar application of SA with concentrations higher than 5000 μM in soybean yielded increases in leaf length and mainly root values. However, this was a field situation, where higher concentrations of SA may be required. In these situations, root growth may be potentiated after treatment with SA, giving the plant greater ability to absorb water and nutrients during times of water stress (Kadioglu et al., 2011).

Figure 4 shows the dry mass values for the two cultivars and imbibition methods. As expected, again the tendency that occurred in the previous variables was noticed for the dry mass, because there was a decrease in the fresh mass and length in concentrations higher than 1000 μM and mainly in 5000 μM .

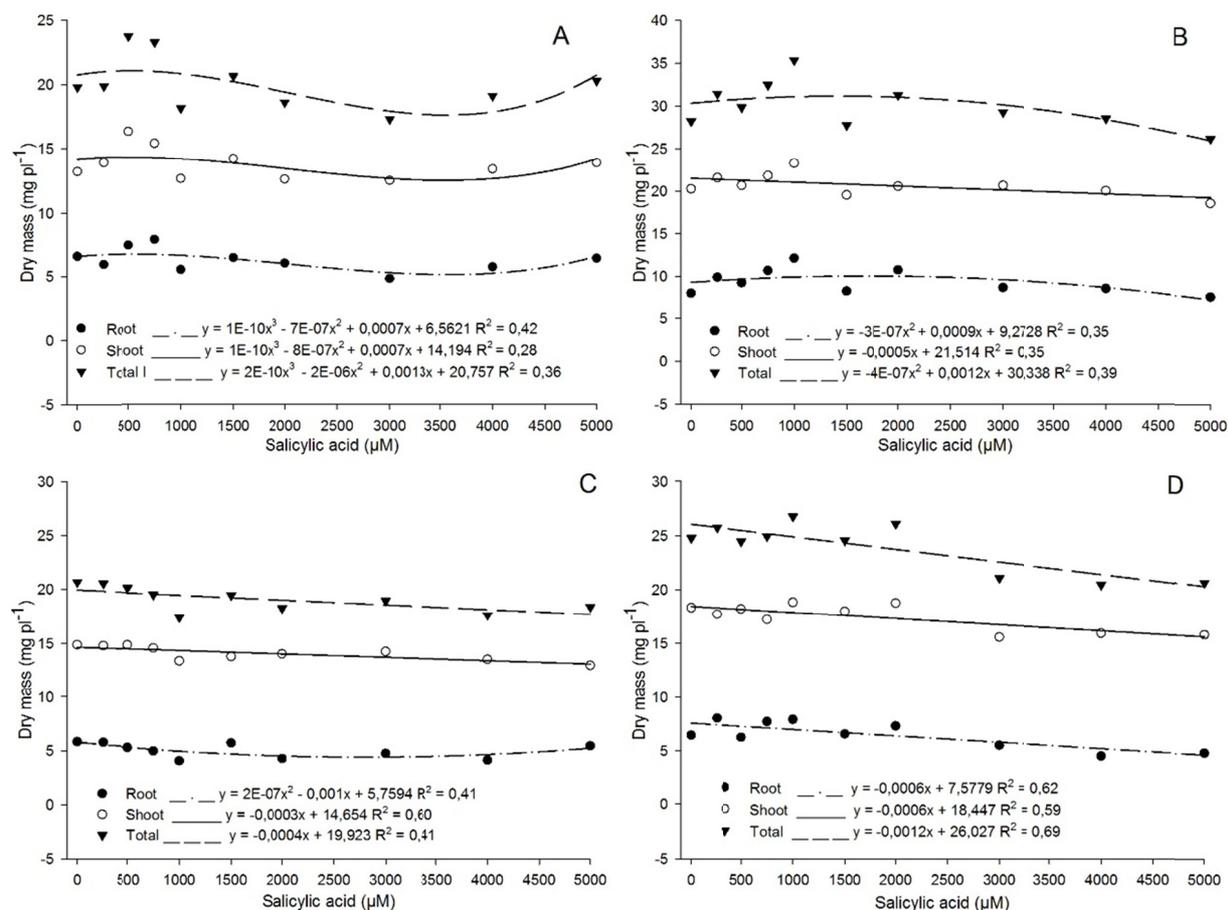


Figure 4. Dry root, shoot and total mass for the cultivars Tec Iriga 6070 RR (A) paper, (B) gerbox and NA 5909 RG (C) paper and (D) gerbox

Therefore, for the dry mass in the two methods in both cultivars the result was similar, and the concentrations between 250 and 7000 µM showed the best results, being these values higher than the control group in many cases. Differently, Al Sahil (2016) did not detect a significant increase of fresh, dry mass and seedling length with the use of SA in the concentrations of 0.5 and 1 ppm, only in the association with gibberellin for the percentage of germination under saline stress in seeds of cucumber. Similarly, Mazraei, Ganjalil, and Rad (2016) did not reach significance for the parameters of thousand seed weight and wheat yield using the concentrations 900, 1800 and 2700 µM.

Lisboa et al. (2017) observed that for different sweet sorghum cultivars, there was drastic reduction of parameters such as germination, germination speed index, number of leaves, length and dry mass of shoot and root in concentrations ranging from 5000 to 20000 µM. This suggests that it is not necessary to use concentrations above 5000 µM and that the use of SA concentrations in the range between 250 and 1000 µM can be used in soybean aiming less damage in face of different kind of stresses. In addition, only 24 hours of imbibition in the gerbox method showed an increase in most variables evaluated. For the paper method, there were also variables with increase in values, however, the values are smaller when compared to the time of 24 hours of imbibition of the seeds in gerbox. These results corroborate with Gupta, Meena and Datta, (2017) which used 500 µM of SA and observed the increase of germination, root length and aerial part, fresh and dry mass of soybean under heavy metal stress.

The method of application of SA also showed interference. In this context, positive results were found by Kuchlan, Kuchlan, and Husain (2017), where SA foliar application provided higher productivity, sanity, germination, and vigor of soybean seeds during storage. Similarly, the same methods used by Fernandes et al. (2019) in the bean culture showed that 24 hours of imbibition, as opposed to the germination paper, up to 1000 µM provided positive results for germination and other variables analyzed. In fact, this concentration range proved to be the most adequate in the evaluated parameters, serving as the basis for the work and according to

the literature.

Therefore, future work should be carried out in an attempt to elucidate the parameters of different species, including in the soybean crop. Many of these studies show that there is a concrete effect on the antioxidant activity against biotic and abiotic stresses, and extensive studies are already underway to determine role of this plant hormone by molecular technics (Kang, Li, & Guo, 2014).

Thus, it is suggested that concentrations above 1000 μM impair the evaluated parameters, and the range between 250 and 750 μM can be used in future studies with soybean seeds to evaluate the behavior under stress. In addition, the gerbox method for 24 hours provided the best results without drastic decrease of the parameters in lower concentrations of SA.

4. Conclusions

Concentrations of SA between 250 and 750 μM can be used in soybean seeds, however, above 1000 μM , the use of this compound may impair physiological quality parameters.

The gerbox method for 24 hours provided the best results without drastic reduction of the parameters in the lowest concentrations of SA.

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