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Salicylic Acid and Its Effect on Physiological and Photosynthetic Parameters in Soybean Seedlings Under Water Deficit

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Abstract

The objective of this study was to evaluate the photosynthetic and physiological parameters of soybean plants under water deficit after imbibition in different concentrations of salicylic acid during germination. The initial seed quality of the cultivar Bayer®/Tec Irga 6070 RR was evaluated. The soybean seeds were soaked in 25 ml in the salicylic acid solution (SA) for 24 hours and the retention capacities of 30, 50 and 70% were adopted. Under controlled conditions, concentrations of zero, 250, 500, 750 and 1000 µM, evaluating the variables length, fresh and dry mass of root and shoot. In the greenhouse, the concentrations of SA of zero, 500 and 1000 µM were used. After 29 days of the seedling, the stomatal conductance, length, fresh and dry mass of root and shoot were evaluated. The results showed that the uptake of SA in the germination aided seedlings under water deficit. The retention capacity of 30% simulated the water deficit, damaging the physiological parameters of soybean seedlings in both environments. The concentrations of 500 and 1000 µM of SA were efficient in the water deficit for the variables root length, fresh and dry shoot mass.

Keywords: conductance, stomata, enzyme, drought

1. Introduction

Soybean production has global economic importance in agribusiness, and, in order to guarantee safety and production increase, it is necessary to use different techniques and management practices. In addition to these aspects, the beginning of a crop will have its uniformity determined by the use of seeds with high physiological potential, among other aspects (Marcos Filho, 2015b). However, biotic and abiotic stresses may decrease productivity throughout the growing season (Danquah et al., 2014; Jaleel et al., 2009; Morando et al., 2014).

Among the most common stresses, water deficit is considered one of the major causes of crop losses, especially in critical stages of development, such as germination and seedling establishment (El Sabagh et al., 2015). This stress promotes morphological, physiological and biochemical changes resulting in oxidative stress and formation of reactive oxygen species (ROS) (El Sabagh et al., 2019).

In order to aid in this process, the plant hormone salicylic acid (SA) plays an essential role in relation to biotic and abiotic stresses, an aspect explored by different researches. Still, SA is responsible for important biochemical and physiological processes, such as growth and development, stomatal closure and nutrient absorption (Hayat, 2010; Janda et al., 2014; Miura & Tada, 2014; Vazirimehr & Rigi, 2014; Parmoon et al., 2017).

The seed has become a vehicle where products and technologies are applied in order to increase soybean productivity. The application of SA has already been used with promising results, however, it is evident that there are gaps to be filled, since the results are variable, according to the species, cultivar, and type of stress observed (Fahad et al., 2017). Therefore, the objective of this study was to evaluate the photosynthetic and physiological parameters of soybean plants under water deficit after imbibition of different doses of salicylic acid during the germination of seeds.

2. Material and Methods

The work was conducted at the Didactic and Seeds Research Laboratory (LDPS) of the Plant Science Department of the Federal University of Santa Maria, Santa Maria, RS. Commercial soybean seeds of cultivar Bayer®/Tec Irga 6070 RR produced in the 2015/2016 crop was used, which is recommended for cultivation in the state of Rio Grande do Sul.

Initial evaluation of seeds: samples from Tec Irga 6070 RR cultivar were evaluated 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 (Nakagawa, 1999; Brasil, 2009).

Evaluation of physiological parameters in soybean seedlings under water deficit on controlled conditions: the seeds were submitted to soaking in gerbox boxes under three germination papers moistened with 25 mL of salicylic acid solution (SA) (Sigma-Aldrich®). The concentrations of SA on the solutions were: zero, 250, 500, 750 and 1000 μM. The seeds remained soaked for 24 hours in BOD under constant light and temperature of 25 ºC. After this time of imbibition, the seed samples were transferred to plastic cups containing 350 g of sand, where sand retention capacities were determined (30, 50 and 70%) to simulate the water stress, according to Brasil (2009).

Four replicates of 3 seeds were sown in each glass, which was kept in BOD, under continuous light, and at 25 ºC, and the amount of water required to maintain their retention capacity was supplied daily, according to Brasil (2009).

During this period the germination speed index was performed, and on the eighth day after sowing the seedlings were removed from the cups with sand and washed for the evaluation of length, fresh and dry mass of root and shoot, without the cotyledons (Nakagawa, 1999).

Evaluation of physiological and photosynthetic parameters in soybean seedlings under water deficit in greenhouse: the seeds were submitted to imbibition in gerbox-type boxes under three germination paper moistened with 25 mL of salicylic acid solution (AS) (Sigma-Aldrich®). The concentrations of SA on the solutions were: zero, 500 and 1000 μM. The seeds remained soaked for 24 hours in BOD under constant light and temperature of 25 ºC. After this time of imbibition, the seeds were transferred to vessels containing 1800 g of substrate, where sand retention capacities were determined (30, 50 and 70%) to simulate the water deficit, according to Brasil (2009).

A series of six replicates of three seeds were carried out in each pot, being these in a greenhouse and random distributed, in order to avoid variations caused by temperature and sun position. Daily, water was supplied in order to maintain the retention capacity, according to Brasil (2009).

After the period of 29 days when the plants were in the phenological stage V2-3 (2 or 3 leaves-varying according to the treatment) an evaluation of the stomatal conductance of water vapors (Gs-mol H_2O m^{-2} s^{-1}) in the developed leaves of two plants (Portable System of Photosynthesis LCi-SD (Fehr & Caviness, 1977). After this evaluation, the plants were removed from the substrate and washed for evaluation the length, fresh and dry mass of the root and shoot (Nakagawa, 1999).

Statistical analysis: the sand experiment was conducted in a completely randomized design (RD), with four replications per treatment, each replicate being composed of three plants. In the greenhouse experiment also RD used, being composed of six replicates per treatment, each replicate being composed of three plants. Initially, the assumptions of the mathematical model were verified by the software Assistat (Silva & Azevedo, 2009). In case of non-compliance with the normality of the errors and homogeneity of the variances, the data were transformed by the methodology $\sqrt{x}$. For the two experiments the data were submitted to variance analysis by the F test ($p < 0.05$) and comparison of means ($p < 0.05$) through the software Sisvar® (Ferreira, 2011).

3. Results and Discussion

The physical and physiological characterization of the Bayer®/Tec Irga 6070 RR soybean seeds cultivar is presented in Table 1. It can be observed that there is adequate humidity and the germination rate was above 80%. The data of the initial characterization was not submitted to statistical analysis, once the objective of this evaluation was only to characterize the physiological quality of the cultivar (Marcos Filho, 2015a).

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 Tec Irga 6070 RR

TSW	Moisture	FC	G	RL	SL	RDM	SDM	EC	FE	ESI
g	------------- % -------------			------ cm -----		-------- mg --------		iS cm^{-1} g^{-1}	%	
137	12.97	83	92	7.6	14.9	87	198	55.8	97	14.3

Table 2 presents the results concerning the variables of length, fresh and dry mass of root and dry mass of shoot in the experiment under controlled conditions. There was no interaction between the factors, therefore, they were analyzed separately. In the retention capacity of 70%, which simulated water excess, the variables length and root dry mass decreased considerably. In the same way, Wijewardana, Reddya, and Bellaloui (2019) verified that some physiological parameters, chemical and quality factors were affected by water excess.

Table 2. Mean root length (RL) (cm), fresh root mass (FRM), root dry mass (MDR) (g) and dry shoot mass (DSM) (g) as a function of the water retention capacity (%) and salicylic acid (AS) concentrations (µM) under controlled conditions

Retention Capacity	RL	FRM	MDR	DMS
30	8.03 a	0.33 ^{ns}	0.055 a	0.036 ^{ns}
50	8.36 a	0.37	0.051 a	0.050
70	6.39 b	0.35	0.019 b	0.036
Salicylic Acid	RL	FRM	MDR	DMS
0	6.78 b	0.27 b	0.025 b	0.026 ^{ns}
250	7.13 b	0.30 b	0.037 a	0.030
500	8.28 a	0.32 b	0.046 a	0.060
750	8.24 a	0.41 a	0.055 a	0.037
1000	8.27 a	0.43 a	0.046 a	0.045
CV (%)	10.94	10.76	22.74	32.03

Note. * Means followed by the same letter do not statistically differ between each other by the Scott-Knott test ($P > 0.05$).

In environments where there is moisture restriction or excess, the root is one of the first organs to signal its occurrence, after all, the roots provide nutrition and water for all processes. Other structures, as well as the aerial part and leaves are structures responsible for managing evapotranspiration, regulating the balance between soil, plant and atmosphere (Taiz & Zieger, 2004; Silva et al., 2019).

As for SA, it is noticed that between the concentrations of zero up to 1000 µM there was a tendency to increase the values of the variables evaluated in higher concentrations of this plant hormone. These differences were evident in the root variables, with the lowest values being in the control that did not receive SA. Thus, it is seen that the compound does not harm the plant, but rather assists in the processes, as reported by Al-Hakimi (2006), and Kabiri, Farahbakhsh, and Nasibi (2012), who obtained positive results using SA at concentrations of 600 µM and between 500 and 1000 µM, respectively.

Table 3 shows the results of the interaction between SA and retention capacity for the variables length and fresh mass of shoot. These results demonstrate that under controlled conditions the efficiency of SA in the different treatments was not clearly evident. For most of the evaluated variables, the different retention capacities, as well as the concentrations of SA, did not present statistically relevant results, since different studies demonstrate that the SA aided in stress conditions. As reported by Mahmood, Sajid, and Khilji (2018) in seeds and maize plants under salt stress, they obtained the best results in the concentration of 500 µM AS.

Table 3. Average shoot length (SL) (cm) and fresh shoot mass (FSM) (g) as a function of water retention capacity (%) and salicylic acid concentrations (µM) under controlled conditions

Salicylic Acid	Retention Capacity					
	SL			FSM		
	30	50	70	30	50	70
0	12.29 ns B	13.54 B	12.67 B	0.32 ns ns	0.28 ns ns	0.21 A
250	12.29 ns B	11.77 B	15.36 A	0.32 ns	0.32	0.22 A
500	14.25 ns B	16.35 A	12.03 B	0.30 a	0.34 a	0.06 bB
750	17.83 ns A	14.57 A	16.21 A	0.35 a	0.35 a	0.24 aA
1000	14.97 ns B	17.16 A	18.10 A	0.31 b	0.43 a	0.22 bA
CV (%)	8.71			13.95		

Note. * Means followed by the same uppercase letter in the column and lowercase case letter on the line do not statistically differ between each other by the Scott-Knott test ($P < 0.05$).

Table 4 represents the results found for the variables fresh and dry root mass and stomatal conductance in the greenhouse experiment. As there was no interaction for these variables, the factors were analyzed separately. In this case, the retention capacities of 30 and 70% were detrimental to the variables of weight and root length. The retention capacity of 50%, not interfered negatively in the values, which suggests that this would be the closest percentage to the field capacity conditions.

Table 4. Mean fresh root mass (FRM) (g), root dry mass (RDM) (g) and stomatal conductance (SC) as a function of water retention capacity (%) and salicylic acid concentrations (µM) at greenhouse

Retention Capacity	FRM	RDM	SC
30	2.50 b	0.19 b	0.06 b
50	3.28 a	0.26 a	0.08 a
70	1.24 b	0.10 c	0.06 b
Salicylic Acid	FRM	RDM	SC
0	2.03 ns	0.15 b	0.07 a
500	2.33	0.18 b	0.05 b
1000	2.66	0.22 a	0.08 a
CV (%)	28.57	26.56	28.62

Note. * Means followed by the same letter do not statistically differ between each other by the Scott-Knott test ($P > 0.05$).

When 1000 µM of SA was used, there was an increase in root dry mass and an increase in stomatal conductance (Gs), indicating its positive effect on these variables. According to Miura and Tada (2014), SA is important in the process of stomatal closure, which supports the results obtained.

This assessment may be influenced by many factors such as the solar radiation of the moment, time, or even leaf size. These factors may still be related to the initial vegetative stage of plants with young leaves. However, specific studies of the involvement of SA in these physiological and photosynthetic processes have shown that its effect is beneficial and may be important signaling of stress along with antioxidant enzymes and other compounds on soybean (Ardebili, Iranbakhsh, & Ardebili, 2019).

When the variables length, fresh and dry mass of shoot and root length were evaluated, it was observed that there was a decrease in the values in all treatments in the control without SA in the retention capacity of 30 and 70%, which were detrimental to the development of the seedlings (Tables 4 and 5). Still, it is noted that the concentrations of 500 and especially 1000 µM provided positive results when the plants were under stress. Similar results were found in the study conducted by Arivalagan and Somasundaram (2015) in sorghum, in which plants under water deficit had variables such as length, fresh and dry mass of root and shoot, and photosynthetic pigments improved with the application of 1000 µM SA.

Table 5. Average shoot length (SL) (cm), fresh shoot mass (FSM) (g), dry shoot mass (DSM) (g) and root length (RL) as a function of the retention capacity of water (%) and concentrations of salicylic acid (SA) (μM) in greenhouse

SA	Retention Capacity											
	SL			FSM			DSM			RL		
	30	50	70	30	50	70	30	50	70	30	50	70
0	23.79 bB	49.29 a ^{ns}	21.87b^{ns}	2.79 bB	10.32a^{ns}	1.67 b ^{ns}	0.65 bB	1.74 a ^{ns}	0.28 b ^{ns}	9.57 bB	29.19 a ^{ns}	7.60 b ^{ns}
500	28.76 bB	44.69 a	27.20 b	3.79 bB	7.76 a	2.34 b	0.44 bB	1.32 a	0.40 b	17.39bA	29.49 a	10.39 c
1000	45.55 aA	43.47 a	27.61 b	8.69 aA	7.04 a	2.86 b	1.46 aA	1.22 a	0.50 b	19.67bA	26.47 a	11.85 c
CV (%)	14.66			24.45			27.19			14.26		

Note. * Means followed by the same uppercase letter in the column and lowercase case letter on the line do not statistically differ between each other by the Scott-Knott test ($P < 0.05$).

This result is extremely important and clearly shows the positive effect of SA use on the occurrence of water deficit (La et al., 2019). It should be noted that the use of SA in this study was restricted to the process of imbibing the seeds for 24 hours and after it was performed the simulation of water deficit in the retention capacity of 30% of water. Similarly, results of Sharafizad et al. (2013), and Habibi and Abdoli (2013) corroborate the use of the same SA absorption method, with concentrations below 2000 μM demonstrating efficient results.

The compound was not added during the conduction of the assay, either in solution or foliar. Although, other studies evaluate the application of these compounds by supplying solutions with water or foliar application in times of stress (Damalas, 2019; Kareem, Rihan, & Fuller, 2019; Osama et al., 2019).

The use of SA in the seed as a carrier may be an easy and effective way of absorbing the compound in the early stage of seedling growth. The most common means for this is seed treatment, similar to that proposed in the study. Other studies demonstrate similarly, as described by Azeem et al. (2019) in wheat under salt stress, that the concentration of 1000 μM of SA was more efficient than 500 μM in the soaking of the seeds during 12 hours in several evaluated parameters. Still, the different ways to supply this plant hormone can be subject to further studies, which would demonstrate more interesting results that can have practical applications on the crops improving its productivity.

4. Conclusions

The results obtained in this study suggest that the use of SA in imbibing soybean seeds is an efficient method to support seedlings submitted to water deficit.

The retention capacity of 30% simulated the water deficit situation, damaging the physiological parameters of the soybean seedlings in the controlled environment and in the greenhouse.

The concentrations of 500 and 1000 μM of SA were efficient when there was stress due to water deficit in the retention capacity of 30% for the variables root length and length, fresh and dry shoot mass.

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