

## SALICYLIC ACID AS A MITIGATOR OF WATER STRESS IN BLACK OATS SEEDS

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**ABSTRACT** - Oats are a very popular grass used in crop rotation to provide organic matter, green mass and assist in the natural aeration of the soil. Agronomic factors such as acidity correction and soil fertility adjustments can be controlled, but when it comes to climatic factors, cultivation depends on favorable climate conditions for good plant development. For instance, low rainfall occurrences result in decreased percentage of germination and plant development. Water stress affects plant development. Therefore, this study aimed to evaluate the effect of salicylic acid as a water stress mitigator on black oats. The experiment was carried out in the Laboratory of Molecular Biology and Biotechnology of Products and Microorganisms of the University of Parana. Black oats (*Avena strigosa* Schreb) seeds were submitted to different imbibition treatments: without imbibition, soaked in distilled water, and soaked in salicylic acid. Then, they were placed in gerbox boxes previously moistened with 6000 polyethylene glycol solution with different osmotic potentials (0.0; -0.2; -0.4; -0.6 and -0.8 MPa). The experiment was conducted in a completely randomized design with four replicates. Ten days after the experiment, the following parameters were evaluated: germination percentage, germination velocity index, shoot length and radicle length. Salicylic acid at the concentration used in this study promotes a mitigating effect of water stress caused by PEG6000, increasing the germination percentage of black oat seeds.

**Keywords:** *Avena strigosa* Schreb., polyethylene glycol 6000, osmotic potential, germination test.

## ÁCIDO SALICÍLICO COMO ATENUANTE DE ESTRESSE HÍDRICO EM SEMENTES DE AVEIA PRETA

**RESUMO** - A aveia é uma gramínea muito utilizada na rotação de culturas e quando em ótimas condições de desenvolvimento, fornece matéria orgânica, massa verde e auxilia na aeração do solo. Fatores agrônômicos como a correção de acidez e ajustes na fertilidade do solo podem ser controlados, porém quando se trata de fatores climáticos, o cultivo fica dependente de condições de favoráveis a um bom desenvolvimento vegetal, neste sentido, baixa ocorrência de precipitação resulta em diminuição da porcentagem de germinação e desenvolvimento inicial do vegetal. Assim, este estudo teve como objetivo avaliar o efeito do ácido salicílico como atenuador do estresse hídrico em aveia preta. O experimento foi realizado no Laboratório de Biologia Molecular e Biotecnologia de Produtos e de Microorganismos da Universidade Paranaense. Sementes de aveia preta foram submetidas à diferentes tratamentos de embebição: sem embebição, embebidas em água destilada ou embebida em ácido salicílico, e em seguida, dispostas em caixas do tipo gerbox previamente umedecidas com solução de polietilenoglicol 6000 (PEG6000) em diferentes potenciais osmóticos (0,0; -0,2; -0,4; -0,6 e -0,8 MPa). O experimento foi conduzido em delineamento inteiramente casualizado com quatro repetições. Foram avaliados a porcentagem de germinação, índice de velocidade de germinação, comprimento de parte aérea e comprimento da radícula. O ácido salicílico, na concentração utilizada neste estudo, promove efeito atenuante de estresse hídrico provocado por PEG6000, aumentando o percentual de germinação em sementes de aveia preta.

**Palavras-chave:** *Avena strigosa* Schreb., polietilenoglicol 6000, potencial osmótico, teste de germinação.

### INTRODUCTION

Oats are a yearly grass belonging to Poaceae family, Aveneae, *Avena* genus. Such genus comprises several wild cultivated weed species found in six continents (GUTKOSKI and PEDÓ, 2000). The main

cultivated species in Brazil are white oats (*Avena sativa* L.), yellow oats (*Avena byzantina* C. Koch), species that are utilized for two purposes such as forage and grain production; and black oats (*Avena strigosa* Schreb) used as pasture, by itself or in consortium with other forages, and

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as green fertilizer (FLOSS, 1988; MATZENBACHER, 1999). Black oats are resistant to soils with nutrient deficiencies and to low temperatures (15 to 20°C), and have been utilized in consortium to cover the soil or as forage, and are one of the main sources of proteins for cattle during intercrop, and, therefore, they are very important for livestock (VILELA, 2007).

Besides the appropriate cultivation practices, good field conditions are essential for plant productivity and, therefore, water deficiency is consider one of the most limiting abiotic stresses to agricultural crops because it affects the hydric relations and modifies vegetal metabolism (MELO et al., 2010). This phenomenon occurs in great extensions of cultivable areas and its effects are evident in all phenological stages of a plant (FAROOQ et al., 2009). Water availability in the substrate is fundamental for seed germination because this event depends on seed imbibition resulting from a water absorption period related to the water potential in the substrate (SERT et al., 2009). However, not always the soil conditions are optimal as in the case of saline or sodium soils or with water deficit that are natural occurrences in the arid and semiarid regions (GUEDES et al., 2013).

Seeds that respond better to the limiting factor of the water stress can perform better conditions to germination (DIAS et al., 2008). Regarding that, Silva et al. (2009) points out that studies related to the seed germination response to the artificial stress conditions have special importance because they enable the evaluation of tolerance limit for the survival and adaptation of species to the natural stress conditions (GUEDES et al., 2013). In a study carried out by Yamashita and Guimarães (2010), it was verified that the total germination and germination velocity of *Conyza canadensis* and *C. bonariensis* seeds decrease with the water availability in the substrate starting at -0.15 MPa.

In laboratorial studies to simulate water deficit conditions, polyethylene glycol (PEG) has been commonly utilized as an osmotic agent because it is a chemically inert non-toxic compound (TAMBELINI and PEREZ, 1998). In order to simulate low humidity conditions in the substrate (TAYLOR and HARMAN, 1990). More negative water potential in the medium reduce the water flow into the cell up to the extreme point where water diffusion through simplast stops and the germination process is directly affected (SOUZA and CARDOSO, 2000).

Plants under stress conditions increase the production of organic compounds that constitute resistance inductors called vegetal hormones. Among them are auxins, gibberellins, abscisic acid, cytokinins and ethylene. Other compounds that affect the vegetal growth and development have been described and among them is salicylic acid (SA) that acts as a regulator of endogenous growth of phenolic nature and is also related to the resistance to diseases (TAIZ and ZEIGER, 2017). SA plays a fundamental role in the stress tolerance due to its capacity to induce effects of protection in plants submitted to stress by water deficiency (AZOOZ and YOUSSEF,

2010). SA can act reducing the inhibition of stressed seed germination, interferes root absorption, reduces plant transpiration, and can cause foliar abscission (KERBAUY, 2008).

According to Carvalho et al. (2007), when studying the effect of SA on germination and vigor of calendula seeds (*Calendula officinalis* L.) under thermal and water stress, verified that the application of this acids contributes positively to seed germination. Therefore, this study aimed to evaluate the action of salicylic acid as a mitigator of the water stress promoted by PEG 6000 in black oats seeds.

## MATERIAL AND METHODS

The experiment was carried out in the Laboratory of Molecular Biology and Biotechnology of Products and Microorganisms of Paranaense University (UNIPAR), Campus of Umuarama, State of Paraná, Brazil, from May to June 2018. The experiment was installed in a completely random design with a 3x5 factorial arrangements with four replications and 200 black oats seeds (batches with 50 seeds). The seeds were acquired at the Biological Reserve of Perobas, located in the municipality of Tuneiras do Oeste (PR). Seed disinfection tests were not done and the germination protocol followed the recommendations found in Brazil (2009). In this experiment, the factors A and B were adopted and they were: Factor A, imbibition in salicylic acid (SA), imbibition in distilled water (H<sub>2</sub>O), and without imbibition (wI), and factor B was solutions of polyethylene glycol6000 [PEG6000 (0.0, -0.2, -0.4, -0.6 and -0.8 MPa)].

For the germination test, gerbox plastic boxes were utilized where 50 seeds were displayed on germitest paper, previously moistened with distilled water or solutions of PEG 6000 (calculated: paper mass x 2.5). The papers were previously weighed and the mass value was multiplied by 2.5 to obtain water and solution of PEG 6000 to moisten the substrate (BRASIL, 2009). Before being displayed in boxes, the seed were imbibed for 8h in salicylic acid and distilled water according to each treatment. Each experimental unit was kept in a BOD incubator at 20°C with photoperiod control of 12 h/day.

The experiment was monitored daily and the evaluations were done at the 5<sup>th</sup> and 10<sup>th</sup> day after the experimental installation according to the Rules for Seed Analysis (BRASIL, 2009). The germination percentage, germination velocity index, aerial part length, and radicle length were evaluated. The lengths were measured with a millimeter ruler whereas the germination percentage and germination velocity index were calculated according to Labouriau and Valadares (1976) and Maguire (1962). Germination (G) was calculated by Equation 1:

$$G = \left( \frac{N}{100} \right) \times 100 \quad (\text{Equation 1})$$

Where:

G = germination (%) and

Salicylic acid as a...

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N = number of germinated seeds at the end of the test.

The germination velocity index (GVI) was obtained by Equation 2:

$$GVI = \sum \left( \frac{n_i}{t_i} \right) \quad (\text{Equation 2})$$

Where:

GVI = germination velocity index (adimensional),  
 $n_i$  = number of seeds germinated in time "i" and  
 $t_i$  = time after test installation.

Time "i" corresponds to a period of 1 to 63 days, and the germination percentage was determined daily as well as its counting within the considered period. The test to obtain length values of the aerial part and root was done in rolls of germitest paper containing 20 seeds, following the same treatment described for the germination test. Ten seedlings from each treatment were evaluated. The seedlings were randomly sampled and measured with a millimeter ruler (30 cm).

The obtained data were submitted to analysis of variance and when significant for the concentrations, regression was applied whereas for imbibitions, Tukey's test at 5% probability was used. When the interaction was significant, concentration unfolding for each genotype was

carried out, considering regression and imbibitions, by Tukey's test. The analyses were done in the Sisvar statistical program (FERREIRA, 2011).

## RESULTS AND DISCUSSION

One of the most critical periods for plant survival is during germination until seedling emergence. Understanding the mechanisms that allow seeds of some species to germinate under water stress conditions is very important to search and develop seed that are tolerant to this type of stress, aiming to reach better acclimation to this condition, which provides advantages in relation to seeds sensitive to drought (COLMAN et al., 2014).

The results obtained for germination percentage were significant ( $p \leq 0.05$ ) for the imbibition interaction and osmotic potential. Interaction unfolding indicated that the treatments with seed imbibition ( $H_2O$  and SA) did not differ among themselves, differing from treatment without imbibition, which presented lower germination percentage when analyzing the witness. On the other hand, in the treatments submitted to water stress (-0.2, -0.4, -0.6 and -0.8 MPa), a significant difference between both evaluated imbibitions was observed, and that from all the treatments, the one that presented the greatest germination percentage (69%) was the one in which the seeds were kept imbibed in salicylic acid, confirming its mitigating effect. The lowest germination percentage (2%) was observed in the treatments in which the seeds were not submitted to imbibition (Table 1).

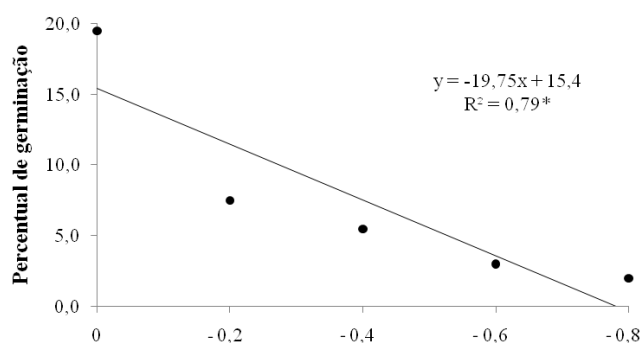
**TABLE 1** - Germination percentage of oats seeds submitted to imbibition treatments and osmotic potentials (MPa).

| Osmotic potentials (MPa) | Germination percentage of black oats seeds (%) |        |        |
|--------------------------|--|--------|--------|
|                          | WI   | $H_2O$ | SA     |
| 0.0                      | 19.5 b*  | 52,0 a | 52,0 a |
| -0.2                     | 7.5 b  | 18,0 b | 54,5 a |
| -0.4                     | 5.5 c  | 20,5 b | 69,0 a |
| -0.6                     | 3.0 c  | 27,5 b | 43,5 a |
| -0.8                     | 2.0 b  | 12,0 b | 48,5 a |
| CV(%)                    | 30.15  |        |        |

\*Averages followed by the same letter in the line do not differ among themselves by Tukey's test, at 5% of error probability, CV = coefficient of variation, WI = without imbibition,  $H_2O$  = water imbibition, SA = imbibition in salicylic acid.

The application of elicitor in plants such as SA guarantees that the treatment promotes an increase in the endogenous levels in the compounds, and that it is enough to generate a response, in this case, a defense response to oxidative damages promoted by water stress. These effects, however, depend on the species, plant development stage, utilized dose and time of exposure to the treatment (HAYAT et al., 2012; ENGELBERTH et al., 2011; AKBULUT, 2014), which showed to be effective when the imbibition methodology was used.

The unfolding of osmotic potential within each imbibition level was significant ( $p \leq 0.05$ ) only for the imbibition treatment. The significant model was the quadratic one, making evident the percentage decrease according to the osmotic potential reduction (Table 1; Figure 1).



**FIGURE 1** - Osmotic potentials on the germination percentage of black oats seeds.

The water deficit is one of the main limiting factors of germination and critical water contents are specific for each species, because each plant type has an external water potential value from which the germination is limited (PEREIRA et al., 2012). Similar results to the ones verified in this study were obtained by Pinheiro et al. (2017), where the germination percentage of melon seeds reduced as water stress severity promoted by PEG6000 increased so that practically there was no seed germination at -0.9 MPa.

Moreover, the utilization of SA as a mitigating substance of the water stress effects on the seed germination, as verified in this study, can be seen as a success considering that there was no statistical difference between the stress levels in this type of imbibition. For seeds submitted to water stress at the most rigorous level (-0.8 MPa), the germination with imbibition in SA was three times greater than water imbibition, and 23 times greater than seed germination without imbibition. Such expressive results for the action of SA as mitigator of the effects of water stress on seed germination have not been found in

the literature. However, Carvalho et al. (2007), in studies on the effect of SA on the germination of calendula seeds submitted to different types of stress, verified a significant difference in the increase of the germination percentage of seeds treated with SA.

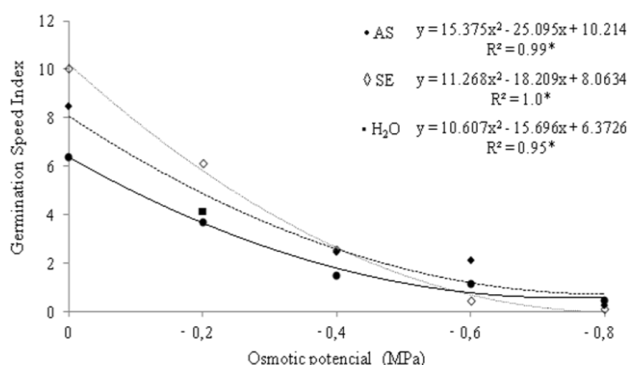
The germination velocity index (GVI) was significant for the interaction of factors ( $p \leq 0.05$ ). The imbibition unfolding within the osmotic potentials revealed that GVI was significant ( $p \leq 0.05$ ) only for the witness and the treatment of -0.2 MPa (Table 2). For the witness, the treatments without imbibition and imbibed in water did not differ among themselves, but they differed in the treatment whose seeds were imbibed in salicylic acid. Although it presented values greater than the germination percentage, SA imbibition presented lower GVI values, indicating that among the treatments was the one with lower germination velocity of seeds. In the stress levels with osmotic potential of -0.2 MPa, the treatment without imbibition surpassed the treatments with water and salicylic acid imbibitions (Table 2).

**TABLE 2** - Germination velocity index of black oats submitted to treatments of imbibition and osmotic potentials (MPa).

| Osmotic potentials (MPa) | Germination Velocity Index (GVI) |                  |         |
|--------------------------|----------------------------------|------------------|---------|
|                          | WI                               | H <sub>2</sub> O | SA      |
| 0,0                      | 10.05 a                          | 8.46 a           | 6.40 b  |
| -0,2                     | 6.14 a                           | 4.12 b           | 3.70 b  |
| -0,4                     | 2.56 ns                          | 2.45 ns          | 1.50 ns |
| -0,6                     | 0.45 ns                          | 2.11 ns          | 1.11 ns |
| -0,8                     | 0.11 ns                          | 0.27 ns          | 0.48 ns |
| CV (%)                   | 32,65                            |                  |         |

\*Averages followed by the same letter in the line do not differ among themselves by Tukey's test, at 5% of error probability, CV = coefficient of variation, WI = without imbibition, H<sub>2</sub>O = water imbibition, SA = imbibition in salicylic acid.

The unfolding of osmotic potentials within the imbibitions was significant ( $p \leq 0.05$ ) and the quadratic model adjust the best (Figure 2). It was observed that for all treatments a decrease in the germination velocity index as the tested osmotic potential became more negative, which represents a more severe stress level.



**FIGURE 2** - Osmotic potentials on the germination velocity of black oats seeds.

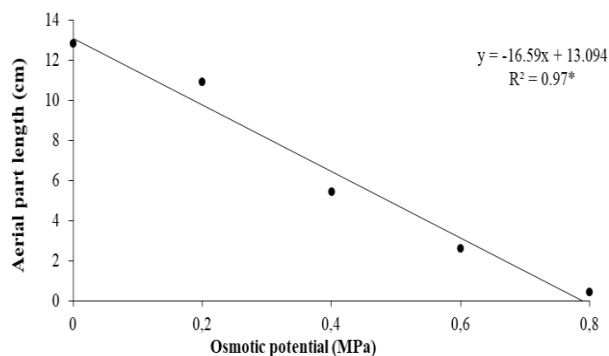
Water stress reduces GVI (ÁVILA et al., 2007) because water scarcity delays the seed germination time (FANTI and PEREZ, 2004) due to the reduction in the

velocity of the metabolic and biochemical processes (PEREIRA et al., 2017). The results obtained in this study are similar to the ones verified by Carvalho et al. (2007) and allow to state that the utilization of SA to mitigate the effects of water deficit causes an increase in the seed germination percentage, but reduces GVI. Likewise, in studies on the germination of chamomile and calendula seeds treated with salicylic acid the same occurred (PACHECO et al., 2007). This can be explained by the fact that SA acts on the protection to water stress, causing metabolic alterations that result in the germination synchronization (TAKAKI and ROSIM, 2000).

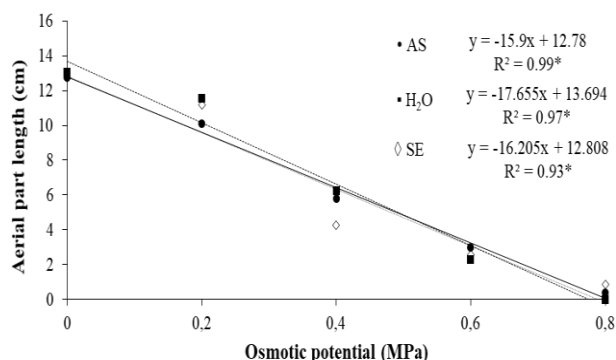
Water scarcity caused reduction of the aerial part length (APL) according to the osmotic potential reduction, and the smaller height was verified in the treatment with -0.8 MPa (0.42 cm), and the greatest (12.85 cm) for the witness (Figure 3), making evident that water scarcity reduces plant height.

The unfolding of osmotic potential within the imbibition factors was significant ( $p \leq 0.05$ ). The model that better adjusted was the linear one, in all interactions. In the treatment without imbibition like in that whose seeds were imbibed in H<sub>2</sub>O or SA, APL was reduced according to the osmotic potential reduction (Figure 4). For the treatment without imbibition, APL varied from 0.86 to 12.74 cm for

-0.8 MPa and witness, respectively. In the imbibition with H<sub>2</sub>O, APL ranged from 0.0cm for the osmotic potential -0.8 MPa to 13.04 cm for the witness, indicating that some seedlings only developed the root system. ON the other hand, the imbibition n SA made it possible to develop seedling with the average values of APL varying from 0.4 cm (-0.8 MPa) to 12.76 cm (witness).



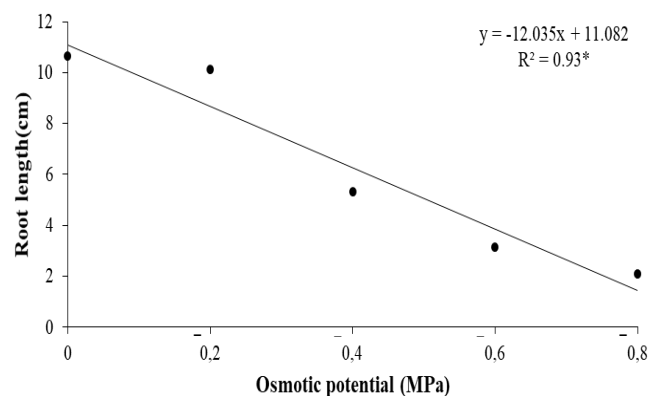
**FIGURE 3** - Osmotic potentials on the aerial part length (cm) of black oats seedlings.



**FIGURE 4** - Osmotic potentials on the aerial part length (cm) of black oats seedlings.

Under conditions of low water availability in the soil, several metabolic processes of the plants can be influenced such as the estomatic closing, estomatic conductance reduction, photosynthesis and transpiration reduction, causing the decrease of the plant growth rate (SILVA et al., 2002; GOMES et al., 2004; PORTES et al., 2006). Taiz and Zeiger (2017) stated that the physiological processes like photochemical transport of electrons, photosynthesis and photoassimilate partition are altered by water stress. For Zhao et al. (2010), these alterations cause decrease of gas exchange, plant size and leaf area.

Similar to what occurred in APL, water stress also reduced the average root length (RL) of seedlings (Figure 5). The unfolding of the imbibition factor on the osmotic potentials was significant only for the potential of -0.4 MPa (Table 3), where a significant difference ( $p \leq 0.05$ ) between the imbibition treatments (H<sub>2</sub>O and SA) was observed, but there was no statistical difference between the treatments with imbibition and the ones without it (Table 3).



**FIGURE 5** - Osmotic potentials on the average root length (cm) of black oats seedlings.

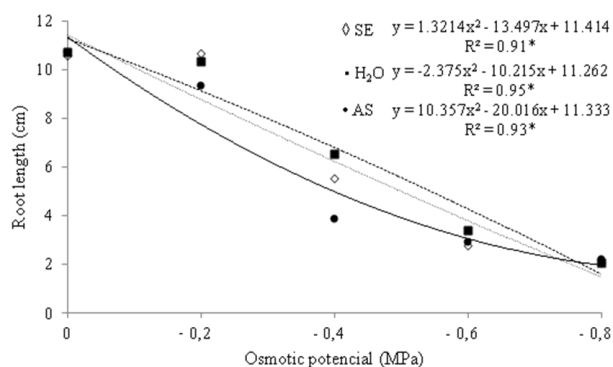
**TABLE 3** - Average root length of black oats seedlings submitted to treatments of imbibition and osmotic potentials (MPa).

| Osmotic potentials (MPa) | Average root length (cm) |                  |        |
|--------------------------|--------------------------|------------------|--------|
|                          | WI                       | H <sub>2</sub> O | SA     |
| 0.0                      | 10.57 ns*                | 10.70            | 10.72  |
| -0.2                     | 10.68 ns                 | 10.33            | 9.34   |
| -0.4                     | 5.50 ab                  | 6.60 a           | 3.87 b |
| -0.6                     | 2.77 ns                  | 3.70             | 2.94   |
| -0.8                     | 2.08 ns                  | 2.05             | 2.20   |
| CV (%)                   | 43.88                    |                  |        |

\*ns = non-significant, averages followed by the same letter in the line do not differ among themselves by Tukey's test at 5% of error probability, CV = coefficient of variation, WI = without imbibition, H<sub>2</sub>O = water imbibition, SA = imbibition in salicylic acid.

The unfolding of the osmotic potential factor on the imbibition was significant ( $p \leq 0.05$ ) for the treatments without imbibition, with imbibition in water and in salicylic acid. For imbibition in SA, the quadratic regression model was the one that best adjusted (Figure 6).

Reduction of the root length was observed according to the reduction of the osmotic potential with values of 10.71 cm for the witness, and 2.20 cm for the most severe stress level (-0.8 MPa).



**FIGURE 6** - Osmotic potentials on the average root length (cm) of black oats seedlings.

The utilization of products to obtain solutions with different osmotic potential and, thus, simulate conditions of low water availability in the soil is very common (MOURA et al., 2011). The use of PEG6000 in osmotic potentials showed that the lower the osmotic potential was, the smaller the growth in the seedlings aerial parts as well as in the root was in the treatments without imbibition and imbibed in water. These results are similar to the ones obtained by Moraes and Menezes (2003) in studies on the performance of soybean seeds under different osmotic potentials and can be explained due to the fact that water restriction modifies cellular turgor and reduces the protein synthesis in the embryo, among others (DELL'ÁQUILLA, 1992).

The effect of SA on AEL and RL were not significant. This can be explained by the fact that SA when applied over the limits tolerated by the plants can cause the reduction of these parameters, as verified by Lisboa et al. (2017), in a study on the influence of SA in saccharin sorghum seeds, where the decrease of these parameters reached values close to 95%. Kerbauy (2008) states that SA can have positive or negative effects in the parameters of plant growth and development, and that these effects depend on the plant interaction with SA and the cultivation medium. This suggests that new studies should be carried out to investigate the interaction between black oats and salicylic acid and the consequences of the plant growth and development as well as its action as an inducer of the plant defense system.

## CONCLUSION

Salicylic acid under the concentration used in this study promotes mitigating effect of the water stress caused by PEG6000, increasing the germination percentage of black oats seeds.

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