Scientia Agraria Paranaensis

**Scientia Agraria Paranaensis – Sci. Agrar. Parana.**
**ISSN: 1983-1471 – Online**

# SALICYLIC ACID AT BEANS GERMINATION AGAINST SALT STRESS

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SAP 20697 Data envio: 01/10/2018 Data do aceite: 30/11/2018
Sci. Agrar. Parana., Marechal Cândido Rondon, v. 18, n. 1, jan./mar., p. 88-92, 2019

**ABSTRACT -** The common bean (*Phaseolus vulgaris* L.) is an important legume that is part of the daily food of the brazilians, being important for other countries. Because it is widely cultivated in almost all brasilian regions, is subject to environmental inclemencies, such as saline stress. Salicylic acid (S.A.) is an important plant hormone known to participate in pathways of systemic acquired resistance (SAR) against pathogens and also reported as a participant in the response to abiotic stresses. This study aimed to evaluate the action of salicylic acid prior to saline stress from three different concentrations of sodium chloride at the germination of a Brazilian common bean, cultivar Avaluna. The experiment was designed in randomized blocks, setting a 2x4 factorial scheme, with eight replicates of 25 seeds each. The following variables were evaluated: percentage of germination, root and shoot length, fresh root and shoot mass. The data were submitted to Tukey test with significance of 5%. The results indicated that there was statistically significant difference in the treatments in relation to the use of AS, that presented stable germination indexes for all evaluated treatments. This result was different from that observed in the treatments with water, where the increase of NaCl concentration resulted in a significant decrease in the percentage of germination. The results also indicated that, for the concentrations of NaCl and S.A. used in the cultivar Avaluna in this experiment, S.A. did not act as a protector against salt stress during the plant growth and development stage evaluated.
**Keywords:** *Phaseolus vulgaris* L., Brazilian bean cultivar, systemic acquired resistance, abiotic stress.

# *ÁCIDO SALICÍLICO NA GERMINAÇÃO DE FEIJÃO FRENTE AO ESTRESSE SALINO*

**RESUMO -** O feijão comum (*Phaseolus vulgaris* L.) é uma leguminosa que faz parte da alimentação cotidiana doss brasileiros, sendo importante para outros países. Por ser cultivada em todas as regiões do país, ela está sujeita a intempéries ambientais, como o estresse salino. O ácido salicílico (AS) é um importante hormônio vegetal conhecido por participar de vias de resistência sistêmica adquirida (SAR) contra patógenos e relatado também na resposta a estresses abióticos. Este estudo teve como objetivo avaliar a ação da aplicação do ácido salicílico anteriormente a um estresse salino, a partir de três concentrações diferentes de cloreto de sódio, na germinação de uma cultivar de feijão brasileira, Avaluna. O experimento foi delineado em blocos casualizados, configurando esquema fatorial 2x4, com oito repetições de 25 sementes. Foram avaliadas as variáveis: porcentagem de germinação, comprimento de raiz e parte aérea, massa fresca de raiz e parte aérea. Os dados foram submetidos ao teste de Tukey, com significância de 5%. Os resultados indicaram que houve diferença estatística significativa nos tratamentos com relação a utilização de AS, pois os mesmos apresentaram índices de germinação estáveis para todos os tratamentos avaliados. Resultado diferente do que foi observado nos tratamentos com água, onde o aumento da concentração de NaCl resultou em diminuição significativa na porcentagem de germinação. Os resultados também indicaram que, para, para as concentrações de NaCl e A.S. utilizadas, o A.S. não obteve atuação como protetor das plantas de feijão cultivar Avaluna, durante os estágios de crescimento e desenvolvimento frente ao estresse salino.
**Palavras-chave:** *Phaseolus vulgaris* L., cultivar brasileira de feijão, resistência sistêmica adquirida, estresse abiótico.

## INTRODUCTION

Common beans (*Phaseolus vulgaris* L.) occupy a prominent place in Brazilian agriculture, being characterized as a strong product at domestic market. IBGE data estimate that the production forecast of the grain for Brazil in 2017/2018 is 3.334 million tons (IBGE, 2018).

Beans are considered a staple food in the Brazilian diet (PONPEU, 1987). Its protein content is so important for populations in developing countries that it was considered an alternative protein source to meat (USDA, 2010).

One of the major problems that hinder the growth of the culture is the frequent exposure to biotic and abiotic stresses. Severe stress can reduce basic food productivity

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by up to 70% (AHMAD et al, 2012), and one of the most devastating stresses affecting agriculture is salinity (IBEKWE et al., 2010).

Saline stress is naturally an edaphic stress that strikes arid and semi arid regions. The origin of salinity problems is mixed with the soil formation itself (LYNCH et al, 2004). In agricultural soils with little drainage, from practices such as irrigation, the increase of minerals such as Na+ and $Cl^-$ in the soil is accentuated, causing saline stress toxicity (ZHU, 2001).

It is estimated that 40% of all food production in the world originates from irrigable soils (MERCHÁN et al., 2018) and therefore, there is a great possibility of accumulation of these minerals in a large number of productive areas. There are controversies about the exact value, however, it is estimated that between 20% and 40% of all irrigated areas suffer from excess salinity (FLOWERS, 1999; CHERAGHI, 2004). In Brazil the problem is found throughout the country, taking place especially in the Northeast, where about 25% of irrigated areas were salted (GHEYI, 2000).

In general, plants suffer from saline stress effects. When plants grow under this stress, the presence of $Na^+$ and $Cl^-$ ions in quantity, limits the absorption of water by the plant, reducing its water potential, causing homeostatic disturbances and, therefore, limiting productivity. Moreover, if these ions enter the plant in excess, these injure the cells responsible for sweating, affecting their growth rates (PARIHAR et al., 2015).

In the germination phase saline stress greatly disrupts yield in several crops (XU et al., 2011; KHODARAHMPOUR et al., 2012). During saline stress, germinating seeds have their metabolism of nucleic acids and protein changed (GOMES FILHO et al., 2008; DANTAS et al., 2007), besides reducing the use of the energy reserves of the seeds, which can lead to the reduction of the germination rate (OTHMAN et al., 2006; SOBHANIAN et al., 2011). The rate of germination decrease is directly associated with saline stress (KAVEH et al., 2011). In beans this is no different. Bayuelo et al. (2002) verified the delay in the germination of several accesses within the species *Phaseolus*.

Salicylic acid (SA) is a hormone known to play systemic acquired resistance action (SAR) (VLOT et al., 2009) against pathogens. Studies also indicate the performance of the SA in response to abiotic stresses, from the exogenous application of the same, such as heavy metals in sunflower (EL TAYEB et al.; 2006), soybeans (DRAZIC et al., 2005; DAT et al., 2000), cucumber (SHI et al., 2006) and maize (HORVATH et al., 2002) and salinity in *Arabidopsis thaliana* (BORSANI et al., 2001) and maize (GUNES et al., 2007).

There are few reports of action in SA bean germination against salt stress. Thus, this work had the objective of verifying the behavior of the common bean cultivar Avaluna, during the germination stage, submitted to salicylic acid and later-to a saline stress.

## MATERIALS AND METHODS

The work was conducted in the premises of the molecular biology laboratory of Universidade Paranaense (UNIPAR), in the city of Umuarama, Paraná, Brazil.

For seeds disinfection, 10% neutral detergente was used, followed by 10 min. wash with 70% etanol. After, a wash with 2.5% sodium hypochlorite for 15 min. was accomplished. Afterwards, the seeds were submitted to 3 washes with autoclaved distilled water.

The seeds were germinated according to the Rules of Seed Analysis - RAS (BRASIL, 2009). For this, they were packaged on 2 sheets of germitest paper and covered with another sheet of the same paper. All seeds were moistened in the proportion of 2.5 times the dry mass (g) of the substrate. The experimental design was a randomized complete block design in a 2 x 4 factorial scheme, with no SA and SA (0.1 mM) and four NaCl doses (0, 50, 100 and 150 mM). Twenty-five seeds were sown with a spacing of 5 cm in length and 4 cm in width, per block. The rolls of paper were packed in a plastic bag. Each treatment consisted of 8 blocks of 25 seeds, totaling 200 seeds per treatment. The seeds were all conditioned in a biochemical oxygen demand type incubator (B.O.D), at a constant temperature of 20 degrees.

The treatments were: 1: control, with distilled water; 2: control, with 0.1 mM salicylic acid; 3: 0.1 mM salicylic acid + 50 mM sodium chloride (NaCl); 4: 0.1 mM salicylic acid + 100 mM sodium chloride (NaCl); 5: 0.1 mM salicylic acid + 150 mM sodium chloride (NaCl); 6: control with 50 mM sodium chloride (NaCl); 7: control with 100 mM sodium chloride (NaCl); 8: control with 150 mM sodium chloride (NaCl).

Treatments 3, 4 and 5, after 24 h immersed in salicylic acid, were submitted to stress with NaCl for 14 days. Treatments 6, 7 and 8, after 24 h of germination in distilled water, were submitted to stress with NaCl for 14 days. After this period, the following evaluations were performed: aerial part length, root length, fresh root weight and fresh weight of shoot.

## RESULTS AND DISCUSSION

Regarding the percentage of seed germination, the results indicate that there was statistically significant difference between the treatments. Seeds submited to AS treatment, obtained stable germination indexes for all evaluated treatments. That was diferente from what it was observed in the treatments with water, where the increase of NaCl concentration resulted in a significant decrease in the percentage of germination evidenced by the high negative correlation between these two variables (r = -0.94*). For SA treatments, the correlation obtained was -0.4671, indicating a weak to moderate linear relationship between the variables (Table 1).

**TABLE 1 -** Iteration table between the germination percentage of the bean cultivar Avaluna submitted to different concentrations of NaCl and salicylic acid (AS).

| | 0 mM NaCl | 50 mM NaCl | 100 mM NaCl | 150 mM NaCl |
|---|---|---|---|---|
| 0.1 mM SA | 171.250 bA* | 186.250 aA | 186.250 aA | 151.250 aA |
| $H_2O$ | 215.000 aA | 210.000 aA | 166.250 aB | 158.750 aB |
| CV(%) | 14.82% | | | |

*Different letters differ statistically from one another. Capitals in the same row and lower case in the same column.

The adjusted polynomial regression between the germination of the treatments in which AS was used and the NaCl doses was the quadratic ($Y = -0.002x^2 + 0.252x + 68.1$), as well as for the treatments without SA ($Y = -0,0001x^2 - 0.155x + 87.5$). The coefficient of determination obtained for the regression equation (with SA) was 0.9758, meaning that 97.58% of the variation in germination is explained by the regression equation. The coefficient of determination obtained for the regression equation ($H_2O$ control) was 0.89893, meaning that 88.93% of the variation in germination is explained by the regression equation (Figure 1).

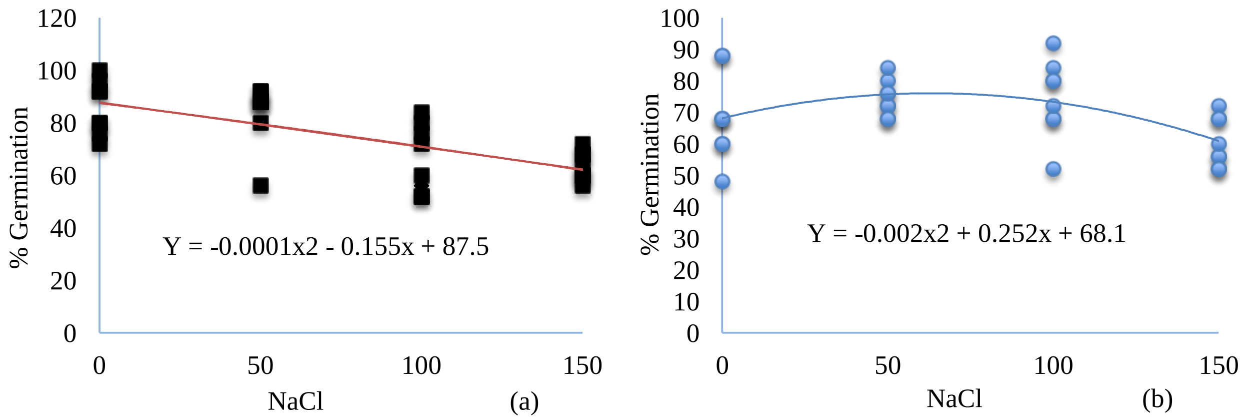


**FIGURE 1 -** Scatter plot and adjusted linear equation for the treatments (a) control (with $H_2O$) and (b) with SA.

During germination, saline stress delays and increases the percentage of non-germinated seeds (RAHMAN et al., 2000). In this way, it was possible to infer that the salicylic acid was able to protect the seeds of the cultivar Avaluna submitted to the posterior salt stress. This is an important result, since the salt stress is one of the most damaging stresses on income sensitive crops. Depending on the concentrations, it reduces plant growth and development, leading to death under severe salinity conditions (SOBHANIAN et al., 2011).

Therefore, this result can be used for further evaluations in other cultivars, because to date, there is no work in the literature that verified the action of salicylic acid against saline stress in Brazilian common bean germination.

In the variable shoot length (Table 2), treatments 0.1 mM SA, 0.1 mM SA + 50 mM NaCl and 50 mM NaCl treatment did not differ statistically from the control treatment. The 0.1 mM SA + 100 mM NaCl treatments and the 100 mM NaCl treatment also did not differ statistically from each other and differed from the control treatment, which had about 46% shorter length than the control. For the treatments 0.1 mM SA + 150 mM NaCl and 150 mM NaCl there was also no statistical difference. These had 67% shorter length than the control being that the latter was statistically equal to the 100 mM NaCl treatment.

In the root length variable, the 0.1 mM SA treatment did not differ statistically from the control. On the other hand, treatments 0.1 mM SA + 50 mM NaCl and 50 mM NaCl did not differ statistically from each other, and were 38% shorter in length than the control. The treatments with 0.1 mM SA + 100 mM NaCl, 0.1 mM SA + 150 mM NaCl, 100 mM NaCl and 150 mM NaCl did not differ statistically from each other, and were 76% shorter when compared to the control.

In the variable Aerial part weight, the 50 mM NaCl treatment was equal to the control treatment, whereas the treatments 0.1 mM SA, 0.1 mM SA + 50 mM NaCl and 100 mM NaCl did not differ statistically from each other and had 19% lower weight than control. The other treatments did not differ statistically from each other and had between 34% and 46% lower weight than the control.

**TABLE 2 -** Length of aerial part and root, shoot weight and root of the bean cultivar Avaluna submitted to different concentrations of NaCl and salicylic acid (SA).

| Treatments | Aerial part length | Root Length | Aerial weight (g) | Root leight (g) |
|---|---|---|---|---|
| Control | 12.38 a* | 14.13 a | 1.29 a | 0.12 ab |
| 0.1 mM SA | 10.68 a | 12.19 a | 1.04 b | 0.09 bc |
| 0.1 mM SA + 50 mM NaCl | 11.28 a | 8.35 b | 0.96 b | 0.1 abc |
| 0.1 mM SA + 100 mM NaCl | 6.57 b | 3.31 c | 0.85 bcd | 0.07 cd |
| 0.1 mM SA + 150 mM NaCl | 4.05 c | 2.16 c | 0.69 d | 0.05 d |
| 50 mM NaCl | 12.12 a | 9.08 b | 1.28 a | 0.14 a |
| 100 mM NaCl | 6.31 b | 3.19 c | 7.16 bc | 0.07 cd |
| 150 mM NaCl | 4.95 bc | 2.17 c | 0.7 cd | 0.04 d |
| CV % | 8.23% | 10.05% | 6.58% | 15.61% |

*Different letters in the same column differ statistically from one another.

In the variable Root weight, the control did not differ statistically from the treatment 0.1 mM SA and 0.1 mM SA + 50 mM NaCl. The variables 0.1 mM SA + 150 mM NaCl and 150 mM NaCl were lower than all other treatments.

The vigor results show that the SA, at the concentration of 0.1 mM, did not act as a protector of the salinity damages in the vigor of the Brazilian bean cultivar Avaluna, when submitted to concentrations of 50, 100 and 150 mM NaCl. All parameters evaluated were severely affected by saline stress. Horvath (2007) argued that protection against abiotic stresses by SA is often not perceived by the plant because there are many variables involved in SA cell signaling.

Although several studies have demonstrated the action of AS as a protector against abiotic stresses, there are reports that in low concentrations (0.05-0.5 mM), as used in this work (0.1 mM), SA may cause oxidative stress in plants (HORVÁTH et al., 2007). This may explain why plants treated with SA only have a lower weight of shoot than control.

This result differs from that found by (AZOOZ, 2009), which used a low concentration of SA (0.2 mM) in two fava bean (*Vicia faba* L.) cultivars submitted to salt stress, and found that AS protected cultivars against this stress, differently in each cultivar. This result shows that the response of SA to saline stress behaves differently in each bean access, as well as in each stage of its development. Therefore, a more detailed study with a greater number of accessions is necessary to clarify if there is a pattern of SA protection behavior against Brazilian bean cultivars. Further studies are also needed to better understand the metabolic pathways involved in the protective action of Brazilian germplasm bean seeds.

**CONCLUSIONS**

It is possible to conclude that the treatment with salicylic acid provided a more stable germination for the seeds of the Avaluna bean cultivar when it was submitted to salt stress.

Depth investigations should be carried out to understand the molecular mechanisms of this protection.

It was also concluded that for the parameters of vigor evaluated, salicylic acid was not efficient for the protection of the cultivar against saline stress.

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