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Salicylic Acid in the Physiological and Sanitary Quality of Corn Seeds

Ácido Salicílico na Qualidade Fisiológica e Sanitária de Sementes de Milho

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Abstract

The exogenous application of salicylic acid has been used as a technique to minimize the harmful effects of salts on plants and improve the physiological quality of seeds. Thus, the objective of the research was to evaluate the effect of different concentrations of salicylic acid on the physiological and sanitary quality of corn seeds. The work was continuous in a completely randomized design, using the hybrid B2315PWU. The treatments were: Treatment 1 (Control), Treatment 2 (500 uM), Treatment 3 (1000 uM), Treatment 4 (1500 uM) and Treatment 5 (2000 uM). The following assessments were carried out: Cold test; first count; germination; seedling length (aerial and root parts); dry mass of seedlings and health test. Salicylic acid did not influence the first count (vigor), germination and cold test. Dry mass increased linearly with increasing salicylic acid concentration. At concentrations up to 1000 uM there was an increase in the aerial and root length of the seedlings. From control to a concentration of 1000 uM, salicylic acid contributes to the incidence of fungi in corn seeds. Physiological variables have a negative relationship with sanitary variables, that is, as the expression of the physiological quality of sensations improved, the incidence of fungi in the seed decreased.
Keywords: *Zea mays* L. Pathology. Physiology. Seedling.

Resumo

A aplicação exógena de ácido salicílico vem sendo utilizada como uma técnica para minimizar os efeitos deletérios dos sais sobre as plantas e como um propulsor da qualidade fisiológica de sementes. Assim, o objetivo da pesquisa foi avaliar o efeito de diferentes concentrações de ácido salicílico na qualidade fisiológica e sanitária de sementes de milho. O trabalho foi conduzido em delineamento inteiramente casualizado, utilizando o híbrido B2315PWU. Os tratamentos foram: Tratamento 1 (Testemunha), Tratamento 2 (500 uM), Tratamento 3 (1000 uM), Tratamento 4 (1500 uM) e Tratamento 5 (2000 uM). Foram realizadas as avaliações: Teste de frio; primeira Contagem; germinação; comprimento de plântulas (parte aérea e radicular); massa seca de plântulas e teste de sanidade. O ácido salicílico não influenciou na primeira contagem (vigor), germinação e teste de frio. A massa seca aumentou linearmente com o aumento da concentração de ácido salicílico. Nas concentrações até 1000 uM houve aumento do comprimento aéreo e radicular de plântulas. Da testemunha até a concentração de 1000 uM o ácido salicílico diminuiu a incidência de fungos em sementes de milho. As variáveis fisiológicas têm relação negativa com as sanitárias, ou seja, à medida que melhora a expressão da qualidade fisiológica das sementes diminui-se a incidência de fungos na semente.
Palavras-chave: *Zea mays L. Patologia. Fisiologia. Plântula.*

1 Introduction

Corn (*Zea mays* L.) is one of the crops of great importance for the world economy and especially the Brazilian one. The Brazilian production of corn grains in the 2022/23 harvest is estimated at 312.5 million tons, an increase of almost 40.1 million tons compared to the previous cycle (Conab, 2022).

Every year, new management technologies are used, from early sowing, the use of fewer seeds per meter and sowing in stumps have become frequent and now require seeds with greater vigor to obtain a good stand and satisfactory productivity (Venkatesh *et al*., 2018). In this context, the application of salicylic acid (SA) in corn seeds was investigated as a potentiator of the seeds' physiological quality.

The exogenous application of salicylic acid has been used as a technique to minimize the deleterious effects of salts on plants. Salicylic acid is a phenolic compound that acts in the signaling and activation of genes that act as plant defense mechanisms against the effects of biotic and abiotic stress (Methenni *et al*., 2018; Silva *et al*., 2018). This compound is a regulator of plant defense mechanisms, acting in the modulation of enzymes that play key roles in physiological and biochemical processes, such as germination, photosynthesis and glycolysis (Nazar *et al*., 2015).

The exogenous application of salicylic acid can act as an inducer of tolerance proteins to different stresses, as well as increase or regulate the activity of cellular detoxification enzymes (Carvalho; Machado-Neto; Custódio, 2007). In this sense, some research has been carried out aiming at the application of salicylic acid in soybean (*Glycine max* (L.) Merril) seeds (Maia; Moraes; Moraes, 2000), in strawberry (*Fragaria x ananassa*) (Trevisan; Madruga-Lima; Zanella-

Pinto, 2017) and in wheat (*Triticum aestivum* L.) (Horváth *et al*., 2007). However, the effect of salicylic acid on seed germination has been controversial, as there are reports suggesting that it can inhibit or enhance seed germination potential. The contradictory results may be related to the concentrations of SA used and the way the product is applied to the seeds (He *et al*., 2010) and there are still few works carried out on the effect of salicylic acid at different concentrations in cut corn.

In this sense, the objective of the research was to evaluate the effect of different concentrations of salicylic acid on the physiological and sanitary quality of corn seeds.

2 Material and Methods

The work was carried out at the Laboratory of Didactics and Research in Seeds and at the Laboratory of Phytopathology Elocy Minussi, at UFSM. The experiment was conducted in a completely randomized design. B2315PWU hybrid corn seeds were used. The sample used was characterized in humidity of 11.35%, germination of 99% and Thousand Seed Weight (PMS) of 417.65 g. Five treatments with different concentrations of salicylic acid were used, namely: Treatment 1 (Control), Treatment 2 (500 uM), Treatment 3 (1000 uM), Treatment 4 (1500 uM) and Treatment 5 (2000 uM). salicylic acid (SA) was diluted in sterilized distilled water, in doses of 0.0900 g L^{-1} - 500 uM; 0.1801 gL^{-1} - 1000 µM; 0.2704 g L^{-1} - 1500 uM and 0.3603 g L^{-1} - 2000 uM. In treatment 1 (Control), only distilled water was used in the defined standard amount. Due to the low solubility of salicylic acid in water, the suspensions were heated in a microwave for 5 min. for complete dissolution.

The seeds were soaked in gerbox boxes on three sheets of germination paper moistened with 25 mL of SA solution at the mentioned concentrations, totaling 50 seeds per box. The seeds remained soaked for 24 h in BOD under constant light and a temperature of 25 ºC. At the end of 24 h, the test assembly began.

The seeds soaked in salicylic acid were submitted to the following evaluations:

First Count (vigor) and germination - the evaluation of the percentage of vigor and germination was carried out in rolls, in four repetitions of 50 seeds, on paper substrate, in a germinator set at 25 °C, soaked in water in the amount of 2.5 times the weight of the dry substrate, aiming at adequate moistening, with counts made on the 4th day (vigor) and on the 7th day (germination) after sowing, according to the Rules for Seed Analysis (Brasil, 2009). The results were expressed in percentage by the average of the repetitions.

Length of the seedlings (shoots and roots) - to evaluate the length of the seedlings, the test was carried out at the end of the first counting test, randomly picking 10 normal seedlings from each repetition, and with the aid of a graduated ruler the aerial and radicular parts were measured and the results expressed in centimeters, according to Nakagawa (1999).

Dry mass of seedlings - the dry mass of seedlings was performed by drying the normal seedlings obtained in the first count. The repetitions of each sample were placed in identified paper bags, and taken to an oven with forced air circulation, maintained at a temperature of 65 °C for a period of 48 h (Nakagawa, 1999). After this period, each repetition had its mass evaluated on a scale with a precision of 0.0001g, with the average results expressed in milligrams per seedling.

Cold test - Performed with four repetitions of 50 seeds for each treatment, in Germitest paper rolls moistened with distilled water in the amount of 2.5 times the mass of the substrate and kept at a temperature of 10 °C for seven days and at 25 °C for four days, evaluating the percentage of normal seedlings at the end of the period (Vieira; Carvalho, 1994).

Sanity test - the sanitary analysis was determined by the filter paper method (Blotter test), described in the Rules for Seed Analysis (BRASIL, 2009b). A sample of 200 seeds was used, divided into eight replications of 25 seeds, placed in gerbox-type boxes, previously disinfected with alcohol (70%) and hypochlorite (1%), under two sheets of filter paper moistened with distilled and sterilized water. Seeds were incubated in a BOD chamber at 25±3 °C for 24 h. Then, to inhibit germination, they were submitted to the freezing method for 24 h. After this procedure, they were incubated at 25 °C for seven days according to the methodology proposed by Rules for Seed Analysis (BRASIL, 2009b). The analyzes were carried out with the aid of a magnifying glass and an optical microscope to observe the morphological structures of the fungi, which were identified at the genus level.

It was evaluated whether the statistical assumptions were met. The results were submitted to analysis of variance (ANOVA), and the effects of salicylic acid on corn seeds were evaluated by the F test ($p \leq 0.05$), with the R CORE TEAM program (2023), as well as by principal component analysis (PCA)

3 Results and Discussion

As the assumptions were met, it was not necessary to transform the data. Table 1 presents a summary of the ANOVA, where the coefficients of variation and which variables had significant differences can be observed.

Table 1 - Summary of the analysis of variance with the mean squares of the variables (ANOVA)

F. V	GL	RAD	AER	MS	VIGF	VIG	GERM	FUS	CLAD	PEN
TRAT	4	35,80*	7,05*	0,016*	36,80^{ns}	15,70^{ns}	1,30^{ns}	140,27*	275,18*	204,44*
ERRO	15	7,08	0,60	0,004	16,46	13,00	2,46	43,39	103,29	74,89
C.V (%)		27,90	18,05	1,93	4,26	3,75	1,59	7,200	40,65	14,36

*Significant ($p \leq 0.05$) by F-test; ^{ns}: No significant difference among treatments; C.V (Coefficient of variation); GL (Degrees of Freedom); RAD (Seedling Root Part Length), AER (Seedling Bud Length), MS (Seedling Dry Mass), VIGF (Cold Test - Vigor), VIG (First Count - Vigor), GERM (Germination), FUS (*Fusarium* spp.), CLAD (*Cladosporium* spp.) and PEN (*Penicilium* spp.).
Source: research data.

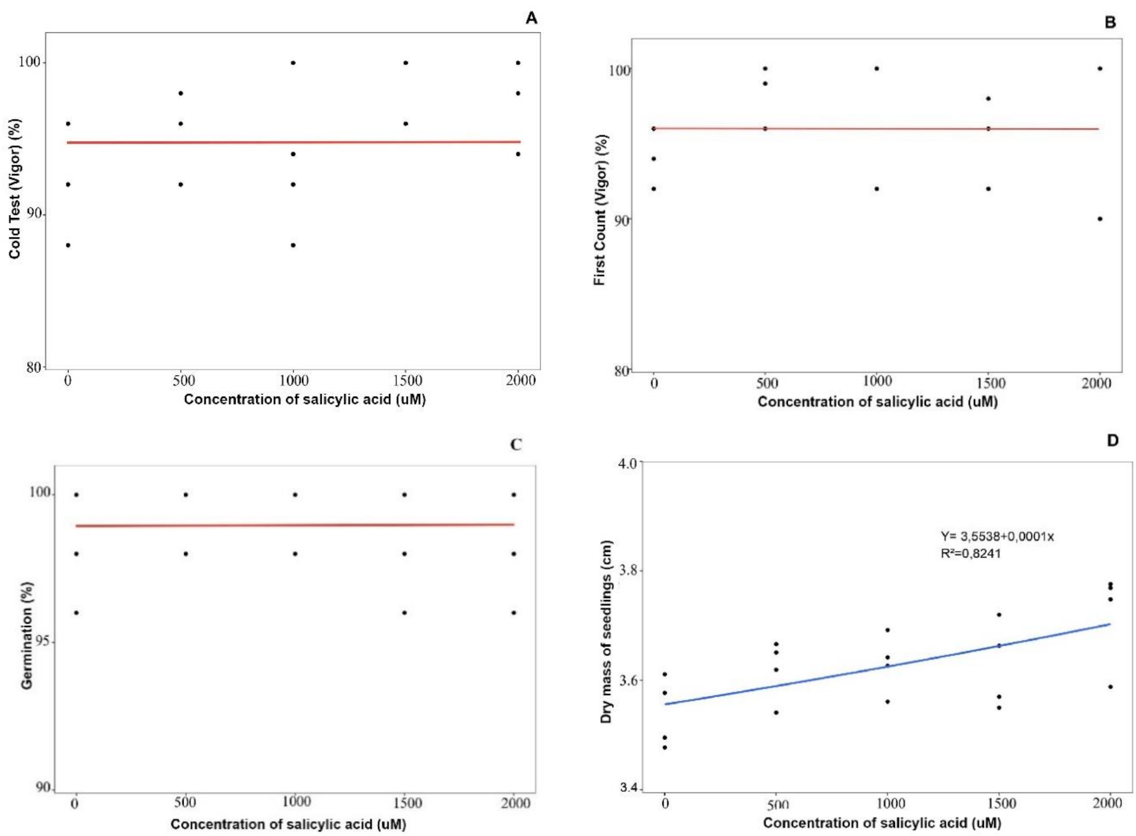
The seeds used, regardless of the use of salicylic acid, have high physiological quality, as it can be seen in Figure 1 (A-C). In the cold vigor test of corn seeds (Figure 1A) there was no statistically significant difference between the concentrations and the control, so that salicylic acid does not influence this quality factor. Exogenous salicylic acid stimulates cell wall lignification, directing the pentose-phosphate and shikimate pathways for the production of phenolic compounds (lignin), forming a cell wall that is more rigid and resistant to biotic and abiotic factors, which contributes to the increase of the vigor of treated plants (Pacheco *et al*., 2007).

There was no influence of salicylic acid on the first count (vigor) (Figure 1B). The exogenous application of salicylic acid aims to develop mechanisms that allow the plant to have better performance and resistance to the effect of stressful conditions (Figueiredo *et al*., 2019; Gomes *et al*., 2018).

For germination, Figure 1C, there was no influence of salicylic acid concentrations in seeds. Maya *et al.* (2000) observed that the germination of soybean seeds was not significantly affected by the presence of SA. Results found by Pacheco et al. (2007) show that concentrations of 0.025 and 0.05 mM of SA favored, but did not synchronize, the germination of marigold seeds (*Calendula officinalis* L.). However, they also verified that concentrations above 0.1 mM impair the germination of chamomile (*Chamomilla recutita* L.) and calendula and inhibit the vigor of chamomile seeds at concentrations equal to or greater than 0.2 mM.

The seedling dry mass results (Figure 1D) show a marked increase in the measure of the increase in salicylic acid concentration in the seed, demonstrated in a linear model. According to the results obtained by Maia, Moraes e Moraes. (2000), for soybean, the weight of green matter is increased, but the dry matter weight of shoots and roots, at doses of 50 and 100 mg kg^{-1}, is reduced by the action of SA.

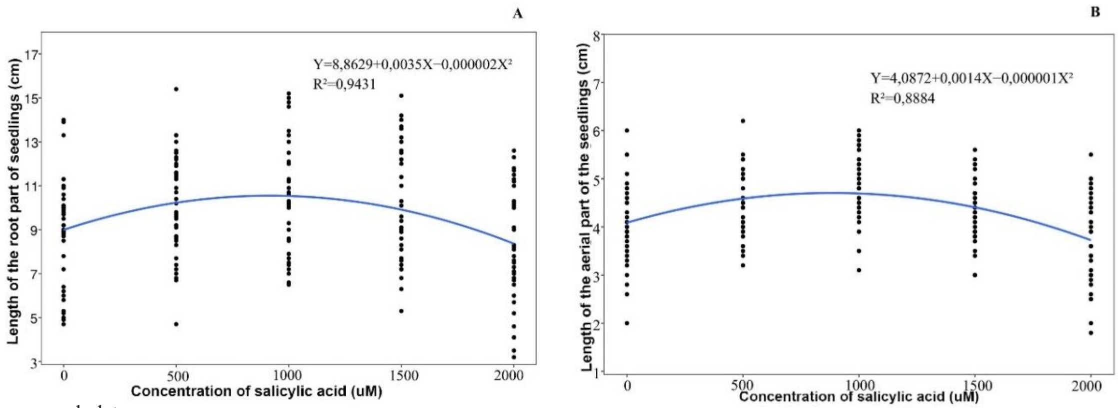
Figure 1 - Cold Test (%; A), First Count (%; B), Germination (%; C) and Dry mass of seedlings (g; D) *Red line (general average) in the variables that did not present statistically significant difference



Source: research data.

The results in Figure 2 for the length of the root and shoot of the seedlings presented in a quadratic model show a significant increase in length up to the concentration of 1000 uM. After this concentration, an antagonistic effect occurs, drastically reducing the development of corn seedlings. SA is a phenolic phytohormone responsible for regulating plant growth and development, demonstrating positive responses under stress conditions (Taiz *et al*., 2017), in addition to inhibiting germination and growth under normal conditions. This may explain why at higher concentrations the size of the plants decreased.

Figure 2 - Length of the root part of the seedling (cm; A) and length of the aerial part of the seedling (cm; B)

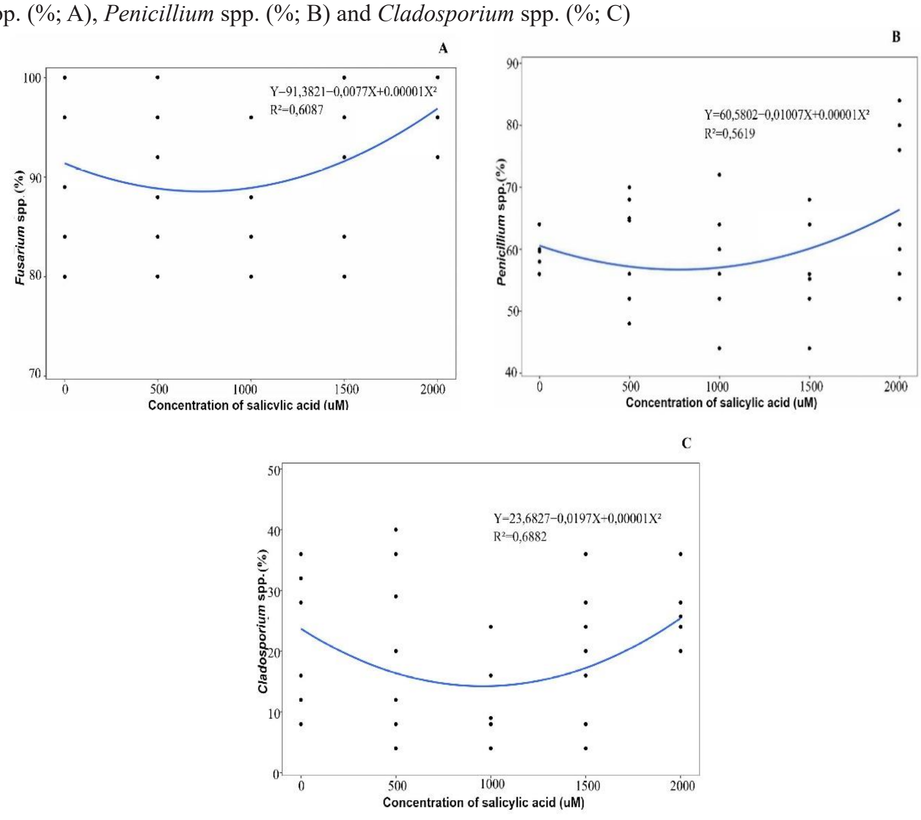


Source: research data.

The results in Figure 3 indicate the incidence of fungi evaluated by the sanity test, where the predominance was observed of the genera *Penicilium* spp., *Fusarium* spp. and *Cladosporium* spp. Seed salicylic acid concentrations showed similarities in responses for different fungal genera. From the control treatment to the concentration of 1000 uM, there was a decrease in the percentage of fungus incidence in the seed. From this point on, there was an increase in fungal contamination at higher acid concentrations.

Bertoncelli *et al*. (2016) evaluating different concentrations of salicylic acid (SA) in inducing resistance to tombing in beet seedlings and antifungal activity against *Fusarium* spp., in vitro found that SA acted to control *Fusarium* spp., in vitro with fungitoxic action, with 28% of mycelial growth compared to the control.

Figure 3 - *Fusarium* spp. (%; A), *Penicillium* spp. (%; B) and *Cladosporium* spp. (%; C)

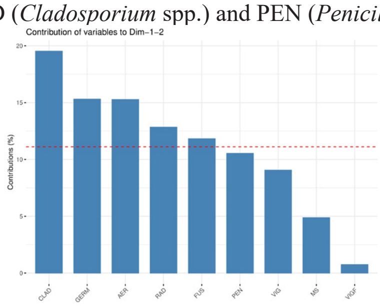


Source: research data.

It was considered interesting to analyze, through a non-parametric analysis, the relationship between the variables under study, since there are physiological and sanitary variables of the quality of maize seeds. With this, Principal Component Analysis (PCA) was used, using the R Core Team (2023) program for this purpose.

Figure 4 presents the variables that most contribute to explain the analysis in 2 dimensions. Thus, it seems that the vigor assessed by the cold test and the dry mass were removed from the analysis to improve the explanatory power of the analysis, as it can be seen in Figures 5A and 5B in the percentage in the two main dimensions.

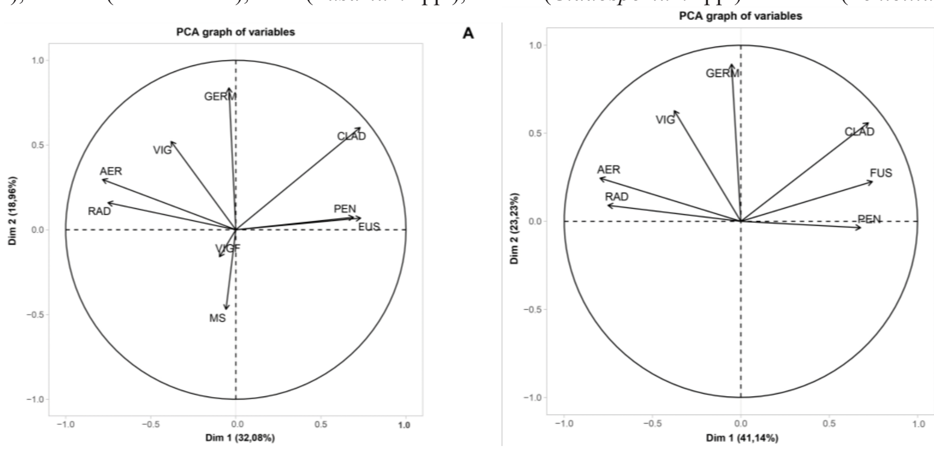
Figure 4 - Contribution of variables to Dim-1-2 of principal component analysis. RAD (Seedling Root Part Length), AER (Seedling Bud Length), MS (Seedling Dry Mass), VIGF (Cold Test), VIG (First Count), GERM (Germination), FUS (*Fusarium* spp.), CLAD (*Cladosporium* spp.) and PEN (*Penicilium* spp.)



Source: research data.

As seen in the principal components Figure 5, there is a positive relationship between the physiological variables of seed quality, due to the proximity and direction of the vectors, as well as because they are in the same quadrant of the plane. The health variables, CLAD, FUS and PEN also present a positive relationship with each other close to what happened between the physiological ones. Between the groups of variables there is an inverse correlation relationship, it can be said that as there is a better physiological quality of the seeds there is a decrease in the presence of fungi in the corn seed, which are health quality factors, generating a negative effect on the relationship among the variables.

Figure 5 - Principal component analysis (PCA) of the study variables, without removal (A) and with removal (B) of low contribution variables in the analysis. RAD (Seedling Root Part Length), AER (Seedling Bud Length), MS (Seedling Dry Mass), VIGF (Cold Test), VIG (First Count), GERM (Germination), FUS (*Fusarium* spp.), CLAD (*Cladosporium* spp.) and PEN (*Penicilium* spp.)



Source: research data.

Principal component analysis aimed to analyze the relationship among the study variables, regardless of the concentration of salicylic acid used. This technique can be used to generate indexes and group individuals. The analysis groups individuals according to their variation, that is, individuals are grouped according to their variances, that is, according to their behavior within the population, represented by the variation in the set of characteristics that define the individual (Hongyu; Sandanielo; Oliveira Junior, 2016).

It is characterized as a non-parametric method, which extracts relevant information from a multivariate data set. The responses obtained via PCA may present patterns or structures that would not be identified if these same data were analyzed by univariate or bivariate methods. Unlike some techniques, in PCA there is no distinction between dependent and independent variables, that is, there is only one set of variables (matrix) to be studied (Yeater; Duke; Riedell, 2015).

4 Conclusions

Salicylic acid did not influence the physiological quality of the seeds, evaluated by the first count, germination and cold test tests. Dry mass increased linearly with increasing concentration. At concentrations up to 1000 uM, there was an increase in shoot and root length of corn seedlings. In the Control treatment and in concentrations of up to 1000 uM, salicylic acid reduced the incidence of fungus in corn seeds. Physiological variables have a positive relationship with each other, just as health variables have a positive relationship with each other. The physiological variables have a negative (opposite) relationship with the sanitary ones, because as the physiological quality improves, the incidence of fungi decreases, regardless of the concentration of salicylic acid used.

References

BERTONCELLI, D.J. et al. Ácido salicílico na indução de resistência ao tombamento de plântulas de beterraba e atividade antifúngica contra *Fusarium* sp., *in vitro. Semina: Ciênc. Agrár.*, v.37, n.1, p.67-76, 2016. doi: 10.5433/1679-0359.2016v37n1p67.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para Análise de Sementes*. Brasilia: Secretaria de Defesa Agropecuária, 2009.

CARVALHO, P.R.; MACHADO-NETO, N.B.; CUSTÓDIO, C.C. Ácido salicílico em sementes de calêndula (*Calendula officinalis* L.) sob diferentes estresses. *Rev. Bras. Sementes*, v.29, n.1, p.114-124, 2007. doi: https://doi.org/10.1590/S0101-31222007000100016

CONAB. Companhia Nacional de Abastecimento. *A produtividade do milho: análise e perspectivas*. Compendio de estudos, v.9, n.9, 2022.

FIGUEIREDO, F.R.A. et al. Respostas fisiológicas de mulungu submetida a estresse salino e aplicação de ácido salicílico. *Irriga*, v.24, n.3, p.662-675, 2019. doi: 10.15809/irriga.2019v24n3p662-675.

GOMES, C.A. et al. Aplicação de ácido salicílico como atenuador dos efeitos de déficit hídrico no milho. *J. Eng. Exact Sci.*, v.4, n.3, p.359-363, 2018. doi: https://doi.org/10.18540/jcecvl4iss3pp0359-0363.

HE, J. et al. Salicylic acid alleviates the toxicity effect of cadmium on germination, seedling growth and amylase activity of rice. *J. Plant Nutr. Soil Sci.,* v.173, n.2, p.300-305, 2010. doi: https://doi.org/10.1002/jpln.200800302

HONGYU, K.; SANDANIELO, V.L.M.; OLIVEIRA JUNIOR, G.J. Análise de componentes principais: resumo teórico, aplicação e interpretação. *E&S Eng. Sci.*, v.5, n.1, p.83-90, 2016. doi: https://doi.org/10.18607/ES201653398

HORVÁTH, E. et al. Exogenous 4- hydroxybenzoic acid and salicylic acid modulate the effect of short-term drought and freezing stress on wheat plants. *Biol. Plantarum,* v.51, n.3, p. 80-487, 2007. doi:10.1007/s10535-007-0101-1

MAIA, F.C.; MORAES, D.M.; MORAES, R.C.P. Ácido salicílico: efeito na qualidade de sementes de soja. *Rev. Bras. Sementes*, v.22, n.1, p.264-270, 2000.

METHENNI, K. et al. Salicylic acid and calcium pretreatments alleviate the toxic effect of salinity in the Oueslati olive variety. *Sci. Horticul.*, v.233, p.349-358, 2018. doi: https://doi.org/10.1016/j.scienta.2018.01.060

NAKAGAWA, J. Testes de vigor baseados no desempenho das plântulas. In: KRZYZANOSKI, F.C.; VIEIRA, R.D.; FRANÇA NETO, J.B. (Ed.). *Vigor de sementes:* conceitos e testes**.** Londrina: ABRATES, 1999. p.2-24.

NAZAR, R. et al. Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline accumulation and ethylene formation under drought stress. *South Afric. J. Bot.*, v.98, p.84-94, 2015. doi: https://doi.org/10.1016/j.sajb.2015.02.005

PACHECO, A.C. et al. Germinação de sementes de camomila [*Chamomilla recutita* (L.) Rauschert] e calêndula (*Calendula officinalis* L.) tratadas com ácido salicílico. *Rev Bra. Plantas Med.*, v.9, p.61-67, 2007.

R CORE TEAM. *A Laguage and Environment for Statistical Computing*, 2019. Disponível em: https://www.r-project.org/.

SILVA, T.I. et al. *Ocimum basilicum L*. seeds quality as submitted to saline stress andsalicylic acid. *J. Agricul. Sci.*, v.10, n.5, p.159-166, 2018. doi:10.5539/jas. v10n5p159

SINGH, P.K. et al. Drought tolerance in plants: molecular mechanism regulation of signaling molecules. In: KHAN, M.I.R. et al. Plant signaling molecules, *Cambridge: Woodhead Publishing*, 2019. p.105-123. doi: 10.1016/B978-0-12-816451-8.00006-X.

TAIZ, L.; ZEIGER, E.; MOLLER, I.; MURPHY, A. *Fisiologia e desenvolvimento vegetal*. 6ed. Porto Alegre: Artmed. 2017, 888p.

TREVISAN F.; MADRUGA-LIMA, C.S.; ZANELLA-PINTO, V. Ácido Salicílico no desenvolvimento de plantas e nas caracteristicas físico-químicas de frutas de morango "Milsei-Tudla". *Rev. Iberoam. Tecnol. Postcosecha,* v.18, n.2, p.106-114, 2017.

VENKATESH, B. et al. Efeito do recobrimento de sementes com fungicidas juntamente com polímero na capacidade de armazenamento de sementes de soja (*Glycine max* L. Merrill). *Int. J. Chem. Stud.,* v.6, n.5, p.598-602, 2018.

VIEIRA, R.D.; CARVALHO, N.M. *Testes de vigor em sementes*. Jaboticabal: FUNEP, 1994.

YEATER, K.M.; DUKE, S.E.; RIEDELL, W.E. Multivariate analysis: Greater insights into complex systems. *Agron. J.,* v.107, n.2, p.799-810, 2015. doi: https://doi.org/10.2134/agronj14.0017