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Physiological and sanitary quality of sorghum seeds under the effect of oxalic and salicylic acid

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Abstract: Sorghum is one of the world's most important cereal crops, but it is susceptible to several diseases, particularly fungal diseases, with seeds being the main vehicle for dissemination. The objective of this study was to evaluate the effects of salicylic acid (SA) and oxalic acid (OA) on the physiological and sanitary quality of sorghum seeds. The seeds *of Sorghum bicolor* (L.) were immersed for 1 hour in solutions of OA (0.5, 1.0, 1.5, and 2.0 mM) and SA (0.5, 1.0, 1.5, and 2.0 mM). A treatment with Captan® fungicide was also used, and the control group was immersed in distilled H2O for 1 hour. Variables from seed vigor tests and fungal incidence tests were evaluated. The control group showed the highest germination rate. However, among the treatments, the doses of 1 mM OA and 0.5 mM SA resulted in higher germination in the first germination count. The application of 1 mM OA and both doses of SA (0.5 mM and 1 mM) promoted a greater seedling vigor index (GVI). Seeds treated with 1 mM OA and doses of 0.5, 1, 1.5, and 2.0 mM SA, as well as those treated with fungicide, had the highest emergence rates, and with 1.5 mM SA, the highest length of the longest root (LVEI). Seedlings from seeds treated with 0.5, 1, and 1.5 mM SA and with 0.5 and 1.5 mM had the highest coleoptile length (CPA). The 0.5 mM dose of SA reduced the incidence of *Aspergillus flavus*, and the doses of 2 mM OA and 1, 1.5, and 2 mM SA reduced the incidence of *Rhizopus stolonifer* to 0%. SA provided the best physiological and health effects for sorghum seeds, making it the best treatment.

Keywords: seeds, endophytic fungi, resistance induction.
Abbreviation: SA_Salicylic Acid; OA_Oxalic Acid; LAFIT_Laboratory Of Phytopathology; EIV_Emergence Velocity Index; GVI_Germination Velocity Index; APL_Aerial Part Length; RL_Root Length; FG_First Germination Count.

Introduction

Sorghum, *Sorghum bicolor* (L.), a prominent member of the Poaceae family, ranks as the fifth most important cereal crop globally and is emerging as a promising feedstock for biofuel production (Zheng et al., 2011). This crop is renowned for its adaptability to arid and semi-arid environments where other crops struggle to thrive (Nida et al., 2019). It is also notable for its growth potential and significant role in agricultural systems in Brazil (Maia et al., 2010).

Sorghum is susceptible to a wide range of diseases, which can restrict its cultivation and diminish yields. Among the diseases affecting the crop in Brazil, the most significant are anthracnose (*Colletotrichum sublineolum*), downy mildew (*Peronosclerospora sorghi*), helminthosporium leaf blight (*Exserohilum turcicum*), rust (*Puccinia purpurea*), ergot or sugar disease (Claviceps africana), and dry rot (*Macrophomina phaseolina*) (Cota et al., 2010; Flavio et al., 2014).

Seeds are considered significant vectors for the dissemination and transmission of numerous microorganisms, with fungi being the most prevalent (Flavío et al., 2014; Cruz et al., 2018). The impact of seed-associated microorganisms varies considerably, primarily depending on the pathogens involved, the amount of initial inoculum, the specific crop grown, and prevailing climatic conditions (Souza et al., 2011).

Seed treatments are essential for controlling pathogens associated with seeds, as well as those inhabiting the soil, storage of fungi, and early leaf pathogens. Under field conditions, effective seed treatment can ensure an adequate plant stand, promote vigorous plant growth, delay the onset of disease epidemics, and increase yield (Moraes, 2010). To reduce the use of chemicals in managing phytopathogens, research has focused on developing efficient alternatives that minimize the harmful effects associated with conventional chemical controls (Moura et al., 2018). Inducing resistance has emerged as a promising alternative, seeking to manage diseases by applying biotic and abiotic agents capable of activating the plants' innate defense mechanisms (Iurkiv, 2008).

Pre-treatment with a non-lethal dose of oxalic acid has been shown to activate defense mechanisms and significantly inhibit fungal growth (Lehner et al., 2008). Jayaraj et al. (2010) demonstrated that the application of oxalic acid enhanced rice resistance to *Rhizoctonia solani* by increasing the accumulation of phenolics and defense-related proteins, thereby offering new strategies for managing this disease in rice crops.

Salicylic acid (SA) is an endogenous plant growth regulator and is part of a diverse group of plant phenolics (Pandey et al., 2013). It was the first plant-derived phenolic compound proven to induce systemic acquired resistance (Araujo et al., 2005). Most phytohormones, including SA, play crucial roles as defensive molecules within signaling pathways, signaling pathogen recognition and activating defense pathways that extend from the site of infection to distal tissues, thus inducing systemic acquired resistance (Vicente & Plasencia, 2011; War et al., 2011; An and Mou, 2011).

Therefore, the objective of this study was to evaluate the effects of salicylic acid and oxalic acid on the physiological and sanitary quality of sorghum (Sorghum bicolor L.) seeds.

Results

Seed germination protocols and conditions

Sorghum seeds untreated (control) exhibited the highest germination percentage at 84%. Lower doses of oxalic acid (OA) at 0.5, 1, and 2 mM, and salicylic acid (SA) at 0.5, 1, 1.5, and 2 mM enhanced seed germination compared to the fungicide treatment and higher concentrations of 1.5 mM OA and 2 mM SA (refer to Table 1). The treatments with 1 mM OA and 0.5 mM SA achieved the highest percentage of seed germination at the first count (FG), recording 43% and 48% respectively, which were comparable to the control. Higher concentrations of OA (1.5 and 2.0 mM) and SA (1, 1.5, and 2 mM) were observed to decrease FG compared to their lower concentrations. The fungicide treatment resulted in the lowest FG (see Table 1). Additionally, the application of 1 mM OA and 0.5 mM SA facilitated a higher initial germination speed (GVI) in sorghum seeds, comparable to the control. In contrast, treatments with fungicide and 2 mM SA resulted in the lowest GVI (refer to Table 1).

Percentage of seedling emergence

Sorghum seeds treated with 1 mM OA and with varying concentrations of SA—0.5 mM (47%), 1 mM, 1.5 mM (62%), and 2.0 mM (45%)—along with those treated with fungicide, exhibited the highest percentages of seedling emergence. Notably, the 1.5 mM concentration of SA achieved the highest emergence velocity index (EVI), with seeds treated with 1 mM SA recording the second-highest EVI (see Table 2).

Seedlings from sorghum seeds treated with SA at concentrations of 0.5 mM (5.41 cm), 1 mM (6.31 cm), and 1.5 mM (5.65 cm) and OA at concentrations of 0.5 mM (6.36 cm) and 1.5 mM (5.35 cm) exhibited the greatest lengths of the aerial parts (APL). The root lengths of the seedlings showed no significant differences across the treatments applied to the seeds (refer to Table 2).

Assessment of fungal incidence on seedlings

The 0.5 mM dose of SA reduced the incidence of *Aspergillus flavus* in sorghum seeds but did not significantly differ from the fungicide treatment. The doses of 2 mM OA and 1, 1.5, and 2 mM SA reduced the incidence of *Rhizopus stolonifer* to 0%, which was comparable to the results achieved with the fungicide treatment. Additionally, seeds treated with 2 mM OA and doses of 0.5, 1, 1.5, and 2 mM SA showed a reduced incidence of *Penicillium* sp., with no significant difference from the fungicide treatment. No concentration of the regulators proved effective in reducing the incidence of *Aspergillus niger* in the seeds. The application of fungicide was the only treatment that successfully reduced the incidence of this fungus.

Statistical analysis using ANOVA for experimental data

All treatments had significant effects on most of the dependent variables, achieving significance at the 1% level ($p < 0.01$). This indicates a very low probability that the observed differences in the means of each treatment occurred by chance. The variable 'APL' (Aerial Part Length) was significant at the 5% level ($p < 0.05$), which still provides strong evidence against the null hypothesis that all treatments have equal effects.

Discussion

Germination is influenced by seed vigor, as observed in seeds treated with 1 mM OA (oxalic acid) and 0.5 mM SA (salicylic acid), which characterize a well-developed plant. The longer it takes for seedlings to emerge from the soil, the greater their exposure to adverse conditions such as the presence of pathogens or inadequate substrate temperatures (Gazola et al., 2013). Additionally, rapid germination is a key component of seed vigor, generally correlating with faster seedling emergence in the field (Marcos Filho, 2015).

The fungicide acts indirectly to increase seedling emergence and the emergence velocity index (EVI); it is not intended to increase seed viability. However, if low emergence is due to a fungal attack, efficient treatment with fungicides will increase these characteristics (Pinto, 2002). Seedlings that emerge rapidly from seeds treated with 1 mM OA, 0.5, 1.5, and 2 mM SA, and fungicides are more prominent than other seedlings with lower development, as they better utilize the resources of the medium (Gustafson et al., 2004).

Seeds treated with SA (salicylic acid) at concentrations of 0.5, 1.0, and 1.5 mM, and OA (oxalic acid) at 0.5 and 1.5 mM

Table 1. Germination (%), first count and germination velocity index (GVI) of sorghum (*Sorghum bicolor*) seedlings treated with different concentrations of oxalic acid (0.5 - OA0.5; 1.0 - OA1.0; 1.5 - OA1.5; and 2 - OA2.0 mM) and salicylic acid (0.5 - SA0.5; 1.0 - SA1.0; 1.5 - SA1.5; and 2mM - SA2.0)

Trataments	Germination (%)	FG (%)	GVI
Witness	84.00±3.46a	42.50±2.60a	16.79±0.82a
Fungicide	60.00±2,89c	17.00±2.89d	10.64±0.84c
OA0.5	67.50±3,75b	34.00±3.46b	13.70±0.84b
OA1.0	75.00±0,58b	43.00±2.31a	15.59±0.36a
OA1.5	58.00±1,15c	32.00±1.15b	12.05±0.26c
OA2.0	71.00±4,62b	28.00±3.46b	13.45±1.05b
SA0.5	76.50±0,29b	48.00±1.73a	15.78±0.58a
SA1.0	72.50±3,75b	27.50±2.60b	14.08±0.83b
SA1.5	69.00±2,89b	32.00±0.00b	13.77±0.52b
SA2.0	59.00±0,00c	24.50±2.02c	11.44±0.25c

Means followed by the same letter in the column do not differ significantly from each other by the Scott-Knott test up to 5% probability. Means ± standard error.

Table 2. Emergence (%), emergence velocity index, aerial part length (APL-cm), root length (RL-cm) of sorghum (*Sorghum bicolor*) seedlings treated with different concentrations of oxalic acid (0.5 - OA0. 5; 1.0 - OA1.0; 1.5 - OA1.5; and 2mM - OA2.0) and salicylic acid (0.5 - SA0.5; 1.0 - SA1.0; 1.5 - SA1.5; and 2 - SA2.0 mM)

Tratament	Emergence (%)	EVI	APL (cm)	RL (cm)
witness	30.00±1.9b	2.75±0.323c	4.51±0.25b	8.63±1.22a
Fungicid	49.00±1.65a	2.68±0.17c	4.55±0.28b	9.18±0.65a
OA0.5	31.00±1.01b	3.02±0.10c	6.36±0.41a	11.11±1.15a
OA1.0	51.00±0.40a	4.98±0.20c	4.61±0.23b	9.80±0.85a
OA1.5	43.00±1.01b	4.15±0.30c	5.35±0.37a	9.88±0.73a
OA2.0	41.00±0.77b	3.08±0.10c	4.44±0.33b	10.08±0.77a
SA0.5	47.00±0.77a	5.31±0.27c	5.41±0.32a	11.26±1.00a
SA1.0	41.00±3.99b	6.36±0.74b	6.31±0.56a	9.71±0.97a
SA1.5	62.00±1.90a	9.62±0.42a	5.65±0.40a	7.44±0.29a
SA2.0	45.00±2.30a	3.41±0.15c	4.61±0.40b	9.31±0.63a

Means followed by the same letter in the column do not differ significantly from each other by the Scott-Knott test up to 5% probability. Means ± standard error.

exhibit increased vigor due to the induction of resistance. This resistance enables them to mobilize reserves from storage tissues to the embryo axis more efficiently, a capability that is reflected in the growth of the seedlings, resulting in higher aerial part lengths (APL) (Marcos Filho, 2015).

The fungi detected in this study are commonly reported in sorghum, as noted by Flavio (2014). The application of SA triggers the induction of response proteins (NPR1) and other defense genes, which enhance plant resistance to pathogens (Yang et al., 2016). This mechanism reduces the incidence of fungal infections. Sub-lethal concentrations of oxalic acid (3 mM) can act as a pre-treatment to combat fungi, with the 2.0 mM dose of OA being notably effective (Lehner et al., 2008). Overall, fungicide treatments are expected to be highly efficient in reducing pathogen presence (Moura et al., 2018). However, alternatives that produce effects similar to fungicides, such as treatments with oxalic acid (2 mM) and salicylic acid (0.5, 1, 1.5, and 2.0 mM), show efficacy against specific fungal species like *Aspergillus flavus, Rhizopus stolonifer*, and *Penicillium* sp.

Fungal attack on seeds reduces vigor by affecting germination (EL-DAHAB et al. 2016). In this study, seeds with high disease incidence (control) had higher germination than seeds with lower disease incidence (treated with fungicides), indicating that the attack by these fungi did not affect seed vigor.

Materials and methods

Plant material

The sorghum (*Sorghum bicolor*) seeds used in this study were acquired from small-scale producers in the municipality of Prata, Paraíba (7°41'35" S, 37°05'22" W), Brazil. These seeds hold cultural significance for the local farmers, often referred to as 'seeds of passion', and have been passed down through generations, from father to son. Following the acquisition, the seeds were transported to the Laboratory of Phytopathology (LAFIT) at the Center for Agrarian Sciences, Federal University of Paraíba. At LAFIT, the seeds underwent a sanitation process involving a 2-minute treatment with 1% sodium hypochlorite. This procedure is critical as it removes surface microorganisms without affecting the seed's internal structure, thus preventing any external fungal contamination from influencing the outcomes of the study focused on seed-borne pathogens.

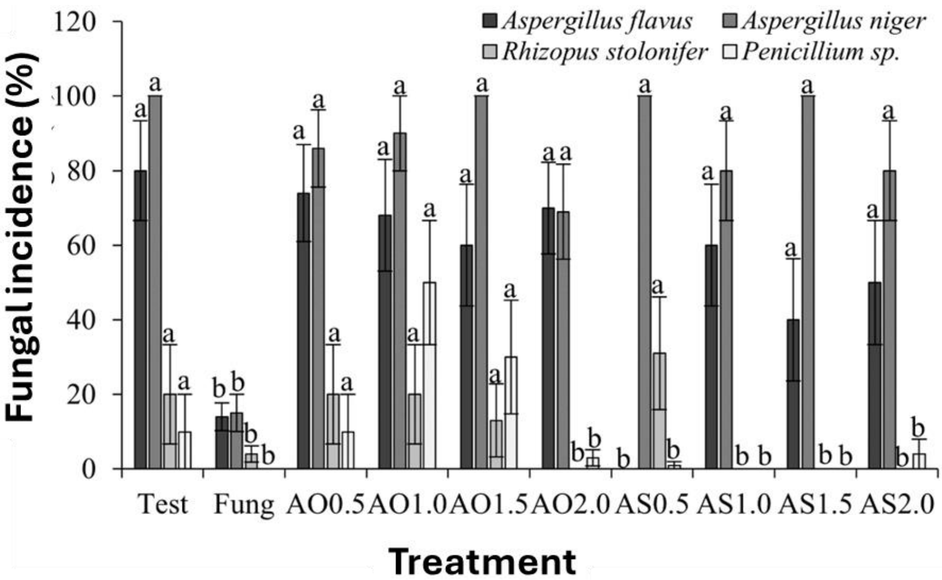

Figure 1. Fungal incidence in sorghum (*Sorghum bicolor*) seeds treated with different concentrations of oxalic acid (0.5 - OA0.5; 1.0 - OA1.0; 1.5 - OA1.5; and 2 - OA2.0 mM) and salicylic acid (0.5 - SA0.5; 1.0 - SA1.0; 1.5 - SA1.5; and 2mM - SA2.0). Columns with the same letter do not differ significantly by the Scott-Knott test up to 5% probability. Vertical bars correspond to the standard error of the mean. Test: Witness; Fung: fungicide

Table 3. Anova of sorghum (*Sorghum bicolor*) seedlings treated with different concentrations of oxalic acid (0.5 - OA0.5; 1.0 - OA1.0; 1.5 - OA1.5; and 2 - OA2.0 mM) and salicylic acid (0.5 - SA0.5; 1.0 - SA1.0; 1.5 - SA1.5; and 2mM - SA2.0).

FV	GL	EVI	GVI	FG	APL	RL	Emerg %	EVI-S	FG-S	RL-S
Treatment	9	15.4871**	283.1667**	356.2333**	4.0018*	5.4871**	352.0**	18.8579**	2.7318**	3.0414*
Error	30	1.8929	31.9667	23.8333	4.1573	4.7392	86.4	2.7765	0.8582	7.399
Mean	-	13.7283	59.7867	44.9783	7.275	8.7517	68.35	4.5353	5.1813	9.6381
CV (%)	-	16.3368	30.4543	27.1093	24.5783	24.275	30.06	56.161	23.327	26.3837

F (**) 1% probability, (*) 5% probability

Experimental design

The experimental design was completely randomized and included 10 treatments. These consisted of four doses of oxalic acid (0.5, 1.0, 1.5, and 2.0 mM), four doses of salicylic acid (0.5, 1.0, 1.5, and 2.0 mM), one fungicide treatment, and one control treatment where seeds were immersed solely in H2O.

Application of treatments

Sorghum seeds were treated with solutions of oxalic acid (0.5, 1.0, 1.5, and 2.0 mM) and salicylic acid (0.5, 1.0, 1.5, and 2.0 mM) for 1 hour, following a modified protocol from Yang et al. (2016). Seeds were also treated with Captan® fungicide at a concentration of 240 g per 100 kg of seed. The control involved immersing seeds in distilled water for 1 hour.

Germination, First count, and germination speed index

For the germination tests, 200 sorghum seeds were divided into four replicates of 50 seeds each and placed between double layers of germination paper, covered with a third sheet, and rolled up. The paper was moistened with water equivalent to 2.5 times its dry weight and placed in a Biochemical Oxygen Demand (BOD) chamber set at an alternating temperature of 20-30°C with an 8-hour photoperiod. Daily counts were performed from the second to the tenth day (Brasil, 2009). Germination results were expressed as percentages. The first germination count (FG) was recorded on the second-day post-sowing, and data were expressed as percentages. The Germination Speed Index (GVI) was calculated daily from the second to the tenth day using Maguire's (1962) formula.

Emergence, emergence speed index, aerial part, and root length

To determine seedling emergence and the Emergence Velocity Index (EIV), 100 seeds were sown in four replicates of 25 seeds each in plastic trays (47x27x8 cm) at a depth of 1 cm, filled with sterilized washed sand. The sand was moistened daily to maintain 60% of its water retention capacity. The trays were placed randomly in a greenhouse, maintained at 25-30°C, and monitored for 10 days (Cruz et al., 2018). Emergence counts were conducted from the fourth to the tenth day, and results were expressed as the percentage of seedlings that emerged by day 10. The Emergence Speed Index was calculated using an adapted formula from Maguire (1962). Measurements of aerial part and root lengths were taken from four seedlings per replicate, totaling 16 seedlings, and 10 days post-sowing.

Fungal incidence

For the fungal incidence study, 100 sorghum seeds were distributed across 10 replicates. Each replicate was placed in a 15 cm diameter Petri dish containing a double layer of moistened filter paper. All materials used were sterilized in an autoclave at 121°C to 134°C for 45 minutes. The Petri dishes were maintained at a constant temperature of 25 ± 2 °C for 7 days. Fungal identification was conducted using stereoscopic microscopy (Cruz et al., 2018), with species verification against specialized literature. Results were expressed as the percentage of infected seeds (Michereff, 2001).

Statistical analysis

Data from the various tests were analyzed using variance analysis (Table 3), and treatment means were compared using the Scott-Knott test at a 5% significance level (Jelihovschi et al., 2014). Analyses were conducted using RStudio version 3.5.1 and Python.

Conclusion

The induction of resistance in sorghum seeds was most effectively achieved with the use of salicylic acid (SA). SA demonstrated superior health and physiological effects on sorghum seeds compared to oxalic acid (OA). We recommend using doses of 0.5 and 1.0 mM of SA for optimal physiological and sanitary quality in sorghum seeds. For OA, a dose of 1.0 mM is advised for enhancing physiological quality, while a dose of 2.0 mM is recommended for reducing fungal incidence.

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