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Physiological quality of seeds from *Passiflora mucronata* Lam. genotypes with nitric oxide donor and salt stress

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Abstract - The presence of salts in the soil solution reduces the osmotic potential, hindering water absorption by roots. However, plants have defense mechanisms against stresses, releasing signaling molecules, in which nitric oxide plays an important role in the abiotic stress. This study aimed to assess the physiological quality of seeds from *Passiflora mucronata* Lam. genotypes pre-conditioned with the nitric oxide donor Tadalafil kept under salt stress. Seeds from nine *P. mucronata* genotypes evaluated under four nitric oxide donor Tadalafil concentrations : 0.0, 1.5, 2.5, 5.0, 7.5, 10.0 and 12.5 mg L^{-1}, applied by soaking seeds for two hours, followed by washing in running water and germination in germitest® paper moistened with NaCl at -1.2 MPa. The experiment was carried out in a completely randomized design, with four replicates of 25 seeds. The nitric oxide donor Tadalafil, at concentrations of 1.5 and 2.5 mg L^{-1}, increased the germination speed, shoot length, and dry mass. Pre-conditioning of seeds with the nitric oxide donor Tadalafil at concentrations of 5.0, 7.5, 10.0, and 12.5 mg L^{-1} determined the death of seeds. Seeds from genotype G3, pre-conditioned with Tadalafil at concentrations of 1.5 and 2.5 mg L^{-1}, presented higher germination, germination speed index, mean germination time, shoot length, root length and dry matter values. Genotypes presented distinct phenotypic responses, providing intraspecific divergence.

Keywords: Germination, Passifloraceae, Passion fruit, Tadalafil, Vigor.

Qualidade fisiológica de sementes de genótipos de *Passiflora mucronata* Lam. com o doador de óxido nítrico e estresse salino

Resumo - A presença de sais na solução do solo reduz o potencial osmótico, dificultando a absorção de água pelas raízes. No entanto, as plantas apresentam mecanismos de defesa contra estresses, liberando moléculas sinalizadoras, nas quais o óxido nítrico desempenha um papel importante no estresse abiótico. Objetivou-se

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com este trabalho avaliar a qualidade fisiológica de sementes de genótipos de *Passiflora mucronata* Lam. pré-condicionados com o doador de óxido nítrico Tadalafil e mantidos sob estresse salino. Sementes de nove genótipos de *P. mucronata* foram avaliadas sob quatro concentrações do doador de óxido nítrico Tadalafil: 0,0; 1,5; 2,5; 5,0; 7,5; 10,0 e 12,5 mg L^{-1}, aplicadas por imersão das sementes por duas horas, seguidas de lavagem em água corrente e germinação em papel germitest® umedecido com NaCl a -1,2 MPa. O experimento foi conduzido em delineamento inteiramente casualizado, com quatro repetições de 25 sementes. O doador de óxido nítrico Tadalafil, nas concentrações de 1,5 e 2,5 mg L^{-1}, aumenta a velocidade de germinação, o comprimento da parte aérea e a massa seca. O pré-condicionamento das sementes com o doador de óxido nítrico Tadalafil, nas concentrações de 5,0; 7,5; 10,0 e 12,5 mg L^{-1}, determina a morte das sementes. As sementes do genótipo G3, pré-condicionadas com Tadalafil, nas concentrações de 1,5 e 2,5 mg L^{-1}, apresentam maiores médias de germinação, índice de velocidade de germinação, tempo médio de germinação, comprimento da parte aérea, comprimento da raiz e massa seca. Os genótipos apresentaram respostas fenotípicas distintas, proporcionando divergência intraespecífica.

Termos para indexação: germinação, Passifloraceae, maracujá, tadalafil, vigor.

Introduction

Passiflora mucronata Lam. (restinga passion fruit) is a wild Passifloraceae, heliophyte, species with climbing habit and herbaceous size, native to the Atlantic Forest, southern coast of Bahia, states of Espírito Santo, Rio de Janeiro, and northern São Paulo, and sandy coastal regions, such as restinga habitats (GARBIN et al., 2012; BERNACCI et al., 2015). This species presents significant potential, and can be used as rootstock for commercial species, in addition to presenting resistance to diseases, such as bacteriosis and anthracnose (OLIVEIRA et al., 2013; SCHMILDT et al., 2018).

High salt concentrations and water deficiency prevent water absorption by seeds, compromising germination due to the reduction in the external osmotic potential or the toxic effects caused by the capture of ions, such as Na^+ and Cl^- (SILVA et al., 2016). Soil salinization, regarding the concentration of soluble salts in the soil solution, occurs mainly in arid and semiarid regions of the world, resulting in plant growth inhibition due to osmotic stress, which restricts water absorption, and ionic stress, in which the accumulating ions in plant tissues causes toxicity due to cellular osmotic imbalance, especially ions Na^+ and Cl^-, in higher frequency. Under stresses, plants present a series of dysfunctions attributed to the reduction of cell turgidity and nutrient absorption, leading to growth reduction due to ionic toxicity, leading to metabolic disorders with morphological, physiological, and molecular alterations (MUNNS et al., 2012; FILIPPOU et al., 2014; LOU et al., 2017).

Salinized soils are mostly found in areas destined for irrigated agriculture, with more significant impact where underground waters are used for irrigation, and the selection of crop varieties is made based on their tolerance to soil salinity (BELTRÁN, 2016; CASTRO; SANTOS, 2020).

Water absorption by seeds characterizes the beginning of germination, which is dependent on the movement of water through tissues that cover the seed, resulting in tissue rehydration and metabolism reactivation, with intensification of the respiratory process and remaining metabolic pathways, promoting the development of the embryonic axis, which results in the protrusion of the primary root (CARVALHO; NAKAGAWA, 2012; BEWLEY et al., 2013). High saline concentrations prevent water from entering the seed, consequently causing reduction in seedling height and root length (GUERRA et al., 2022).

The presence of salts in the soil solution reduces the osmotic potential, reducing wa-

ter absorption by roots (TAIZ et al., 2017). However, plants have defense mechanisms against stresses, releasing signaling molecules, in which nitric oxide (NO), as a redox signaling molecule, plays an important role in physiological growth regulation and plant development processes , in the defense against pathogens, and in the responses to abiotic stress (SANZ et al., 2015; AHMAD et al., 2016).

The exogenous NO donors most used in studies related to plant physiology, aiming at reducing the damage caused by oxidative stress, are sodium nitroprusside (SNP) and S-nitrosoglutathione (GSNO), which are related to plant physiology (KOVÁČIK et al., 2014).

Studies involving other NO donors, such as Sildenafil Citrate, have also been carried out (ZANOTTI et al., 2013; KAISER et al., 2016; MACIEL et al., 2018). Thus, this study aimed to evaluate the physiological quality of seeds from *Passiflora mucronata* Lam genotypes pre-conditioned with the NO (nitric oxide) donor Tadalafil, kept under salt stress.

Material and Methods

The study was conducted at the Laboratory of Seed Analysis (LAS), Center of Agricultural Sciences and Engineering of the Federal University of Espírito Santo (CCAE-UFES), municipality of Alegre-ES. Seeds from nine *Passiflora mucronata* Lam. genotypes (G1 to G9) were used in the experiment, cultivated in a vertical shoot positioning system in the Experimental Area of CCAE-UFES, at geographic coordinates 20º 45`S and 41º 30`W, with mean elevation of 250 m. a.s.l.

Seeds from freshly-harvested ripe fruits were extracted with the aid of sterilized spoon, processed, submitted to aryl removal with calcium hydroxide on nylon sieve, washed in running water, and kept on germitest® paper for 48 hours at 28 ± 3 ºC for drying and adjustment of the water content to 12%.

Subsequently, seeds were pre-conditioned for two hours in 20-mg Tadalafil solutions (Excipients: lactose monohydrate, croscarmellose sodium, hypromellose, sodium laurylsulfate, microcrystalline cellulose, magnesium stearate, magnesium chloride, macrogol, yellow iron oxide dye) at concentrations of 0.0, 1.5, 2.5, 5.0, 7.5, 10.0 and 12.5 mg L^{-1}, followed by disinfestation in 70% alcohol for 1 minute, 2% sodium hypochlorite for 3 minutes, and treated with 5% Captan® for 1 minute.

Salt stress induction was performed using NaCl with osmotic potential of -1.2 MPa, at equivalent ratio of 2.5 times the mass of the dry paper (BRASIL, 2009), after which, seeds were kept in B.O.D. incubator (Biochemical Demand Demand) with temperature alternation (20-30 ºC) and absence of light.

Germination - The count of the number of germinated seeds was daily performed until germination was constant, and was characterized by the protrusion of the primary root with dimension ≥ 2.0 mm. Results were expressed as germination percentage.

Germination speed index - Determined along with the germination test by daily counting the number of seeds that presented primary root protrusion equal to or above two millimeters (MAGUIRE, 1962).

Mean germination time - number of normal seedlings counted in the interval between each count (ni) and the time elapsed between the beginning of germination and the i-th count (ti) as proposed by Labouriau (1983).

Normal seedlings – Determination considers seedlings with all formed structures: root, hypocotyl, and cotyledons (BRASIL, 2009), at 40 days after sowing, with results expressed as percentage.

Shoot length - Measured 40 d after sowing with the aid of a millimeter rule, and the result was expressed as cm $plant^{-1}$.

Root length - Obtained by measuring the distance between the base of the plant and the root tip with the aid of a millimeter rule, and results were expressed as cm $plant^{-1}$.

Fresh and dry seedling matter - Determined at 40 d after sowing with analytical scale

(0.0001 g). After obtaining dry matter, seedlings were stored in kraft paper bags and kept in a forced-air oven at 70 ºC, until reaching constant weight (72 h). Results were expressed as mg seedling^{-1}.

The experiment was carried out in a completely randomized design, in a 9x6 +1 factorial scheme (nine genotypes x six Tadalafil concentrations 1.5, 2.5, 5.0, 7.5, 10.0 and 12.5 mg L^{-1} plus concentration of 0.0 mg L^{-1}), with four replicates of 25 seeds, submitted to salt stress with NaCl at concentration of -1.2 MPa.

For the analysis of variance assumptions to be met, the following data transformations were performed: For germination = [arcsin(x/100)$^{1/2}$], and for the germination speed index = [(x+0.5)$^{1/2}$]. After the transformation of these variables, along with the remaining variables, data were submitted to analysis of variance, and if significant difference was found, means were compared by the Tukey's test at 5% probability, followed by regression analysis. After data transformation, the effects of treatments were tested by analysis of variance, comparing the means by the Scott-Knott test. The original experiment data were presented in the Table for better representativity of the study. For the effect of Tadalafil concentrations, data were submittted to regression analysis, and for the adjustment of equations ($\hat{Y}$), the beta significance was used as a criterion ($p \leq 0.05$). For all analyses, the free R Core Team, 2022.

The genomic DNA (Deoxyribonucleic acid) of the nine *Passiflora mucronata* genotypes were extracted from young leaves of all genotypes according to protocol by Doyle and Doyle (1990), with the following modifications: addition of 1% (w/v) polyvinylpyrrolidone for buffer extraction; washing twice with chloroform-isoamyl alcohol (24:1); and, for precipitation, one third of ammonium acetate (7.5 M) and a volume of cooled isoanol were added. Samples were resuspended in 40 µL of TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA), containing 40 µg mL^{-1} of RNase A. The genomic DNA integrity was analyzed in 0.8% agarose gel stained with ethidium bromide, and the DNA concentration and purity were determined by spectrophotometry.

For analyses with ISSR markers (Inter Simple Sequence Repeat), 40 markers were previously amplified in *P. mucronata* samples, seven of which were chosen for analysis in all analyzed samples. Markers were chosen by the band pattern for being of clear identification and due to its reproducibility. DNA amplifications were performed with ISSR procedures 807, 808, 809, 810, 811, 822, and 834 at the UBC (The University of British Columbia). Each polymerase chain reaction (PCR) contained 30 ng of DNA, 0.5 µM primer, and 1 U of DNA Taq polymerase in a final volume of 13 µL.

Samples were denatured at 94 °C for 5 min followed by 35 cycles of 94 ºC for 1 min, 50 ºC for 1 min, and 72 ºC for 1 min, and final extension at 72 °C for 10 min. PCR products were visualized in 1.5% agarose gel stained with ethidium bromide (1% - 10 mL) and photographed under UV light. Fragments were compared with a 100 bp DNA ladder (Thermo Scientific®).

Based on the genetic information from the seven molecular markers applied to the nine *P. mucronata* genotypes, a dissimilarity matrix was generated with the distance between the pairs of genotypes by the Sorensen coefficient. For e morphological and productive data, the matrices of the generalized Mahalanobis distance were generated. The grouping method, for both cases, was that of the farthest neighbor. The number of groups was determined by the statistical criterion of Mojema, with k=1.25. Statistical analyses were performed using the free R Core Team, 2022.

Results and Discussion

Among the nitric oxide donor concentrations tested, Tadalafil, there was no germination at concentrations of 7.5, 10.0, and 12.5 mg L^{-1}, suggesting that the doses caused germination inhibition in all genotypes. However,

in Tadalafil concentrations of 1.5, 2.5, and 5.0 mg L^{-1}, seed germination was observed.

Seeds from G1, G3, G4, G5, G6, G7, G8, and G9 genotypes presented a parabola-like behavior with upward concavity, and germination increased with pre-conditioning with Tadalafil. However, with the increase in concentration, at 5.0 mg L^{-1}, there was a trend towards the reduction of this percentage (Figure 1). Seeds from G2 genotype presented a decreasing behavior with the use of the NO donor, by which germination tended to decrease.

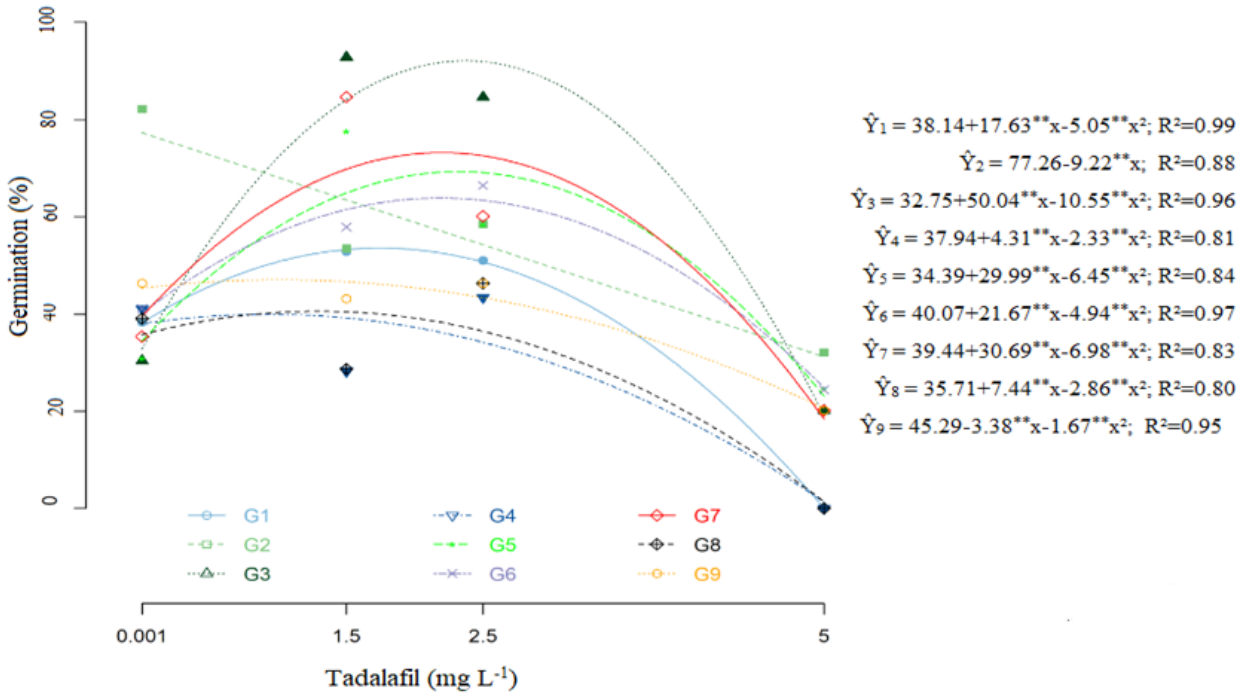

Figure 1 - Germination percentage of *Passiflora mucronata* seeds pre-conditioned with different Tadalafil concentrations under salt stress induced with NaCl at -1.2 MPa. **(p≤0.001); nsnot significant.

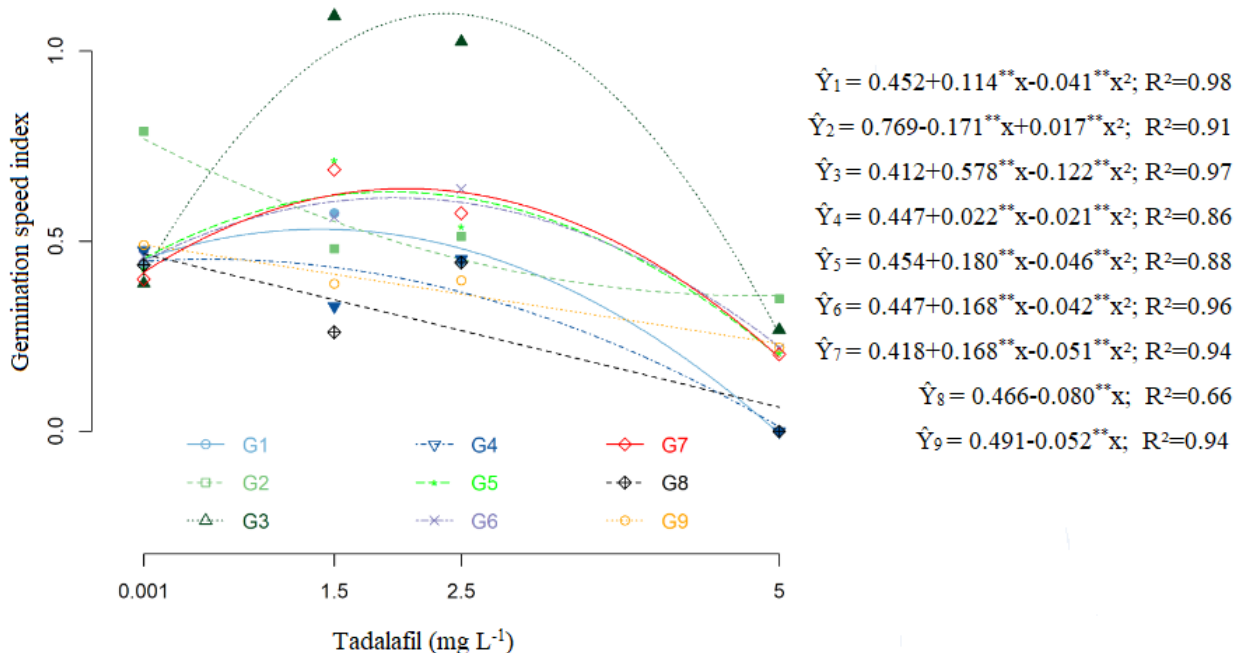

Figure 2 - Germination speed index of *Passiflora mucronata* seeds pre-conditioned with different Tadalafil concentrations under salt stress induced with NaCl at -1.2 MPa. **(p≤0.001); nsnot significant.

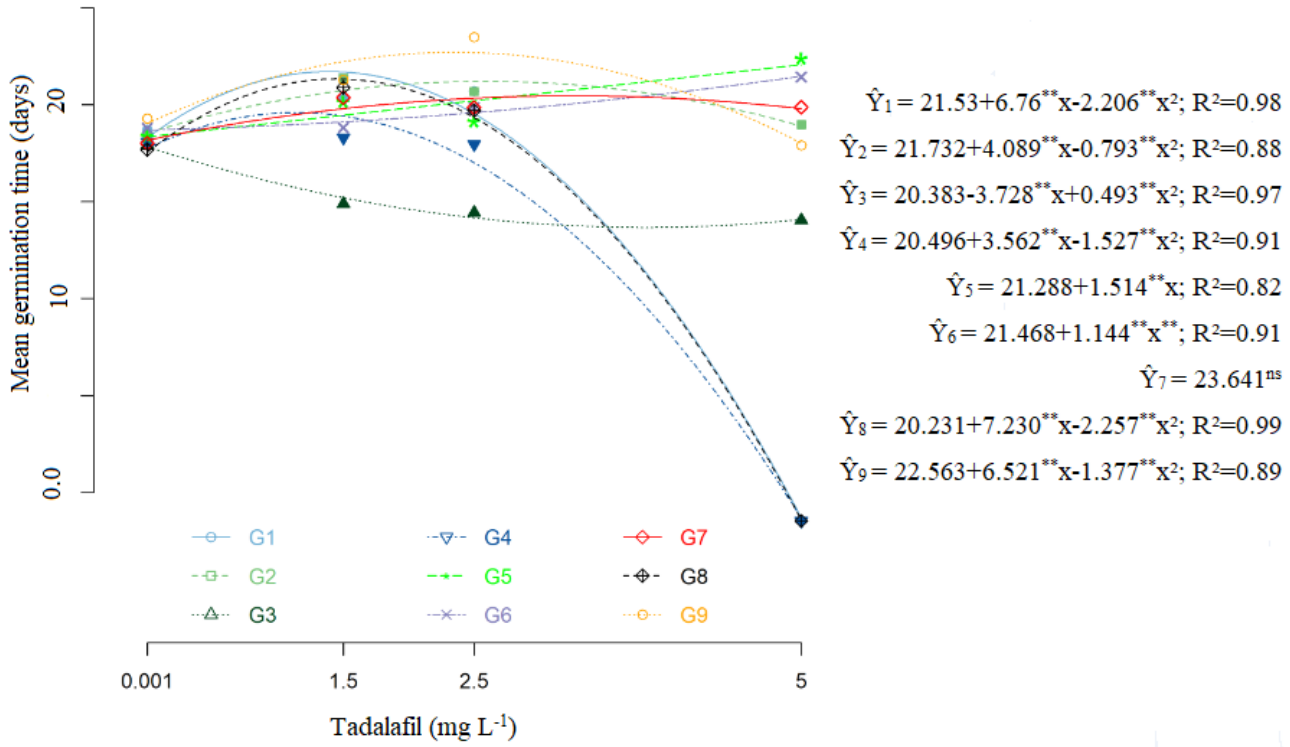

Figure 3 - Mean germination time of *Passiflora mucronata* seeds pre-conditioned with different Tadalafil concentrations under salt stress induced with NaCl at -1.2 MPa. **(p≤0.001); nsnot significant.

There was no germination of seeds treated with Tadalafil dose of 5 mg L^{-1}, suggesting a toxic effect, with the death of G1, G4, and G8 genotypes due to its high reactivity, causing programmed cell death or necrosis (REDA et al., 2018), which suggests the need for the control of the nitric acid homeostasis with dose that reaches the balance between synthesis and degradation.

Seeds from G1, G3, G5, G6, and G7 genotypes, when treated with Tadalafil at concentrations of 1.5 and 2.5 mg L^{-1}, presented increase in the germination speed index (GSI) in relation to control, although there was reduction at concentration of 5.0 mg L^{-1}, forming a parabola (Figure 2), which may be associated to germination unevenness and delayn attributed to the effects of salinity, with reduction in water capture and mobilization of stored reserves, which modify the structural organization of proteins (IBRAHIM, 2016). However, the release of nitric oxide with the application of Tadalafil acts in membranes, improving the imbibition process and providing nutritive media for the growth process and low germination speed index, whereas the dose of 5.0 mg L^{-1} may have caused germination inhibition or delay.

The mean germination time of G3 genotype was reduced, forming a parabola with downward concavity, with the highest reduction observed when treated with nitric oxide donor at concentrations of 2.5 and 5.0 mg L^{-1}, resulting in 14 days (Figure 3). This suggests that the treatment increased and accelerated the germination speed. G1, G2, G5, G6, G8, and G9 genotypes presented increase in the mean germination time when treated with the NO donor at concentrations of 1.5 and 2.5 mg L^{-1}, suggesting that Tadalafil had no effect on the germination acceleration in these genotypes.

The shoot length of seedlings from G1, G4, and G8 genotypes presented an ascending parabola when seeds were treated with Tadalafil, with growing behavior up to the concentration of 2.5 mg L^{-1}, presenting maximum values of 10.4, 10.5, and 11.4 cm respectively, and decreased in treatment with concentration of 5 mg L^{-1}, for not present-

ing seed germination at this concentration (Figure 4). It was observed that G2 and G6 genotypes presented parabolas with growing behavior at concentration of 5.0 mg L^{-1}, with maximum values of 11.7 and 12.2 cm, respectively (Figure 4). Similar behavior was observed in cotton seeds (*Gossypium hirsutum* L.) under salt stress (DONG et al., 2014), possibly due to the fact that nitric oxide increases the osmotic pressure of plant cells and cytoplasm viscosity under these conditions.

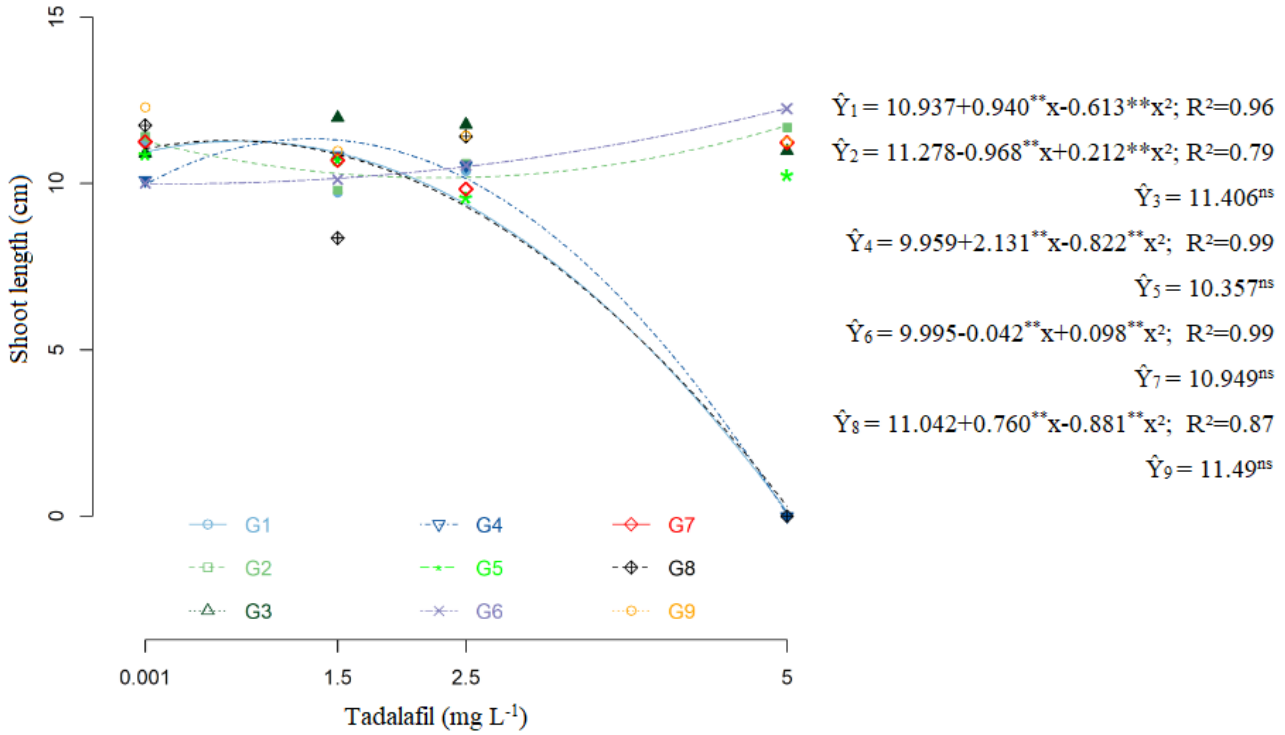

Figure 4 - Shoot length of seedlings from *P. mucronata* seeds pre-conditioned with different Tadalafil concentrations under salt stress induced with NaCl at -1.2 MPa. **(p≤0.001); nsnot significant.

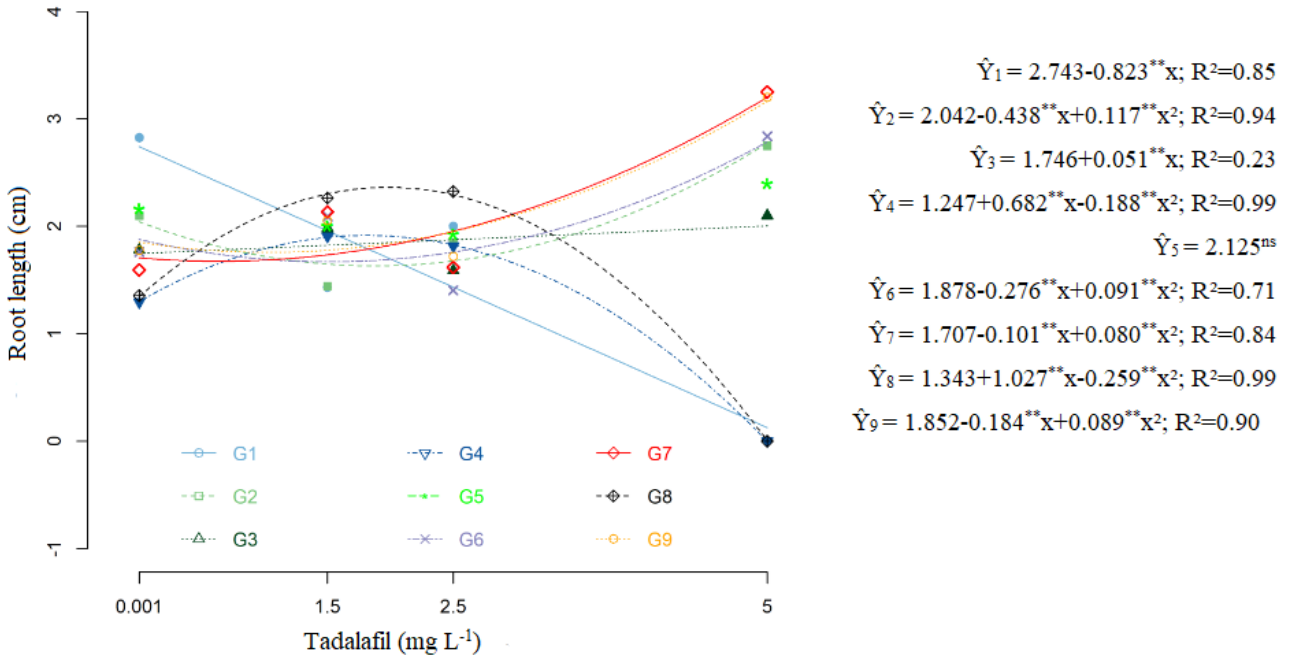

Figure 5 - Root length of seedlings from *P. mucronata* seeds pre-conditioned with different Tadalafil concentrations under saline stress induced with NaCl at -1.2 MPa. **(p≤0.001); nsnot significant.

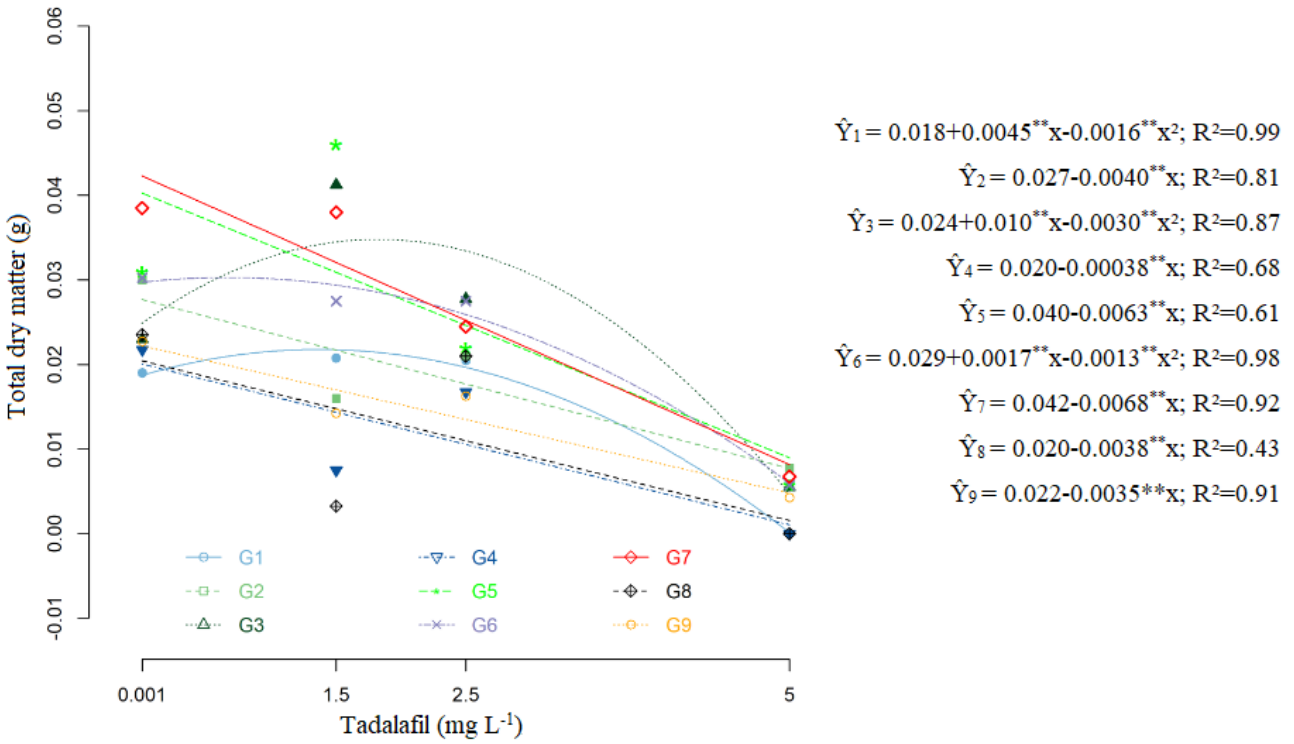

Figure 6 - Total dry matter of seedlings from *P. mucronata* seeds pre-conditioned with different Tadalafil concentrations under salt stress induced with NaCl at -1.2 MPa. **(p≤0.001); nsnot significant.

G1 genotype, treated with the NO donor, presented a decreasing behavior regarding root length, whereas G3 genotype presented a linear growing behavior with maximum value of 2.10 cm at concentration of 5.0 mg L^{-1}, and G4 and G8 genotypes presented radicle increase when treated with doses up to 2.5 mg L^{-1}, decreasing at dose of 5.0 mg L^{-1}, forming a parabola (Figure 5).

By analyzing the total dry matter, it was observed that G1 and G3 genotypes presented increasing parabolic behavior at concentration of 1.5 mg L^{-1}, whereas reduction was observed at concentrations of 2.5 and 5.0 mg L^{-1} (Figure 6). This behavior can be associated with higher germination and higher root and shoot growth of seedlings as a function of the nitric oxide donor, acting at low concentrations in seed membranes, influencing permeability. However, there was reduction of this variable at concentration of 5.0 mg L^{-1} attributed to the death of seeds. G2, G5, G7, G8, and G9 genotypes presented a decreasing behavior with the application of the NO donor.

In the seed germination of genotypes (Table 1) without the application of the nitric oxide donor (Tadalafil) and submitted to salt stress at -1.2 MPa, G2 genotype presented the highest germination percentage (53%), while the others presented values below 20%. Germination is damaged due to the presence of salt, since it inhibits or restricts the water capture by seeds. Due to this restriction, seeds have negative responses, such as dehydration, cytotoxicity, and reduction in the synthesis of new tissues and in their metabolic activity (TAIZ; ZEIGER, 2017; MARCOS FILHO, 2015). However, in the research carried out by Silveira (2021), it was demonstrated that the effective quantum efficiency of the photosystem II was increased by the spraying of NO donors in sugarcane plants under water deficit. Several studies have shown that the manipulation of endogenous NO levels, mainly through exogenous donors, has demonstrated a significant effect on plant tolerance to various stresses, including water deficit.

Table 1 - Germination, germination speed index, mean germination time, shoot length, root length and total dry matter of seedlings from *Passiflora mucronata* genotypes pre-conditioned with different Tadalafil concentrations under salt stress induced with NaCl at -1.2 MPa.

Genotypes	Germination (%)				Germination speed index			
	Tadalafil (mg L^{-1})				Tadalafil (mg L^{-1})			
	0.0	1.5	2.5	5.0	0.0	1.5	2.5	5.0
G1	14b[1]	25d	24c	0c	0.19c	0.33c	0.20d	0.00c
G2	53a	26d	30.5c	10a	0.62a	0.23d	0.27c	0.12a
G3	9d	64a	56a	4b	0.15b	1.19a	1.05a	0.07b
G4	15c	8f	18d	0c	0.23b	0.11f	0.20d	0.00c
G5	9d	49c	30.5c	6b	0.18c	0.51b	0.29c	0.04b
G6	16c	30d	38b	6b	0.21b	0.32c	0.41b	0.05a
G7	12d	56b	32c	4b	0.16c	0.47b	0.33c	0.04b
G8	16c	8f	20d	0c	0.19c	0.07f	0.19d	0.00c
G9	20b	17e	20d	4b	0.24c	0.15e	0.16d	0.05b
CV (%)	6.80				2.16			
Genotypes	Mean germination time (days)				Shoot length (cm)			
G1	22a	25a	26b	0d	11.20a	9.70b	10.40b	0.00b
G2	21a	27a	26b	22b	11.40a	9.80b	10.60b	11.70a
G3	20a	15c	14c	14c	10.90a	12.00a	11.70a	11.00a
G4	21a	21b	21b	0d	10.00b	10.80a	10.50b	0.00b
G5	21a	25a	23b	29a	10.80a	10.70a	9.50b	10.20a
G6	22a	22b	24b	27a	10.00b	10.10b	10.50b	12.20a
G7	21a	25a	24b	24b	11.20a	10.70a	9.80b	11.20a
G8	20a	26a	24b	0d	11.70a	8.30c	11.40a	0.00b
G9	23a	27a	32a	20b	12.20a	11.00a	11.40a	11.20a
CV (%)	6.56				9.34			
Genotypes	Root length (cm)				Total dry matter (mg)			
G1	2.8a	1.4b	2.0b	0.0d	0.02c	0.02d	0.01b	0.00b
G2	2.1b	1.4b	1.8b	2.7b	0.03b	0.02e	0.02b	0.01a
G3	1.8c	1.9a	1.5c	2.1c	0.02c	0.04b	0.03a	0.00a
G4	1.3d	1.9a	1.8b	0.0d	0.02c	0.01f	0.02c	0.00b
G5	2.2b	2.0a	1.9b	2.4c	0.03b	0.05a	0.02b	0.01a
G6	1.8c	2.1a	1.4c	2.83b	0.03b	0.02c	0.03a	0.01a
G7	1.6c	2.1a	1.6c	3.2a	0.04a	0.03b	0.02a	0.01a
G8	1.3d	2.3a	2.3a	0.d	0.02c	0.00g	0.02b	0.00b
G9	1.8c	2.0a	1.7c	3.2a	0.02c	0.01e	0.02c	0.00a
CV (%)	13.33				13.85			

[1]Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other by the Scott-Knott test (p≤0.005).

In treatment with the nitric oxide donor at concentration of 1.5 mg L^{-1}, the highest germination indices were observed in G3, G5, and G7 genotypes, with 64, 49, and 56%, respectively, evidencing the effect of nitric oxide on seed membranes, influencing permeability, which is submitted to the seed water potential and the external medium for the imbibition process to occur, mitigating the toxic effects of salinity to seeds. Similar results were observed in *Dalbergia nigra* (ATAIDE et al., 2015) and *Brassica oleracea* seeds (KAISER et al., 2016).

Germination was not efficient with Tadalafil dose of 5 mg L^{-1} of, which can be explained by the overdose that caused stress to seeds, inducing the death of G1, G4, and G8 genotypes. Although nitric oxide performs several

beneficial effects on seed physiology, when in excess, it may be toxic to plants due to its high reactivity, causing programmed cell death or necrosis (REDA et al., 2018), being necessary to control the nitric acid homeostasis , reached by the balance between synthesis and degradation.

The germination speed index (Table 1) was higher in G2 genotype (0.620) without seed treatment. At concentration of 1.5 mg L^{-1}, superior results were obtained with G3 (1.192), G5 (0.510), and G7 genotypes (0.475). At concentration of 2.5 mg L^{-1}, the highest index was presented by G3 genotype, with 1.052. The germination speed index in treatment with 5 mg L^{-1} was higher than that of G2 genotype, with 0.122. These results evidence the action of nitric oxide in reducing the harmful effects of NaCl during seed germination, increasing the germination percentage with the application of doses of 1.5 and 2.5 mg L^{-1}.

When analyzing the mean germination time (Table 1) in controls, genotypes did not differ statistically from each other, with close indices after 21 days. However, in treatments with 1.5 and 2.5 mg L^{-1}, the genotype that presented the lowest mean germination time was G3 (15 days). Treatment with the nitric oxide donor reduced by seven days the mean germination time. Studies have shown that salt stress negatively affects the germination speed index and root length due to the harmful effects of NaCl on plant establishment (REN et al., 2020). Inhibition of germination and plant growth under salt stress may be related to the inhibition of water absorption by seeds, delaying or inhibiting germination (ALSAEEDI et al., 2017).

Considering the shoot length of seedlings (Table 1) at concentration of 5 mg L^{-1}, although seeds presented reduction in the germination percentage, those that germinated resulted in more vigorous seedlings, especially those of G2, G3, G5, G6, G7, G8, and G9 genotypes, a fact that can be associated to the action of NO, which acts by increasing plant tolerance to environmental stress, including salt stress (LIN et al., 2012; FAN et al., 2014; GADELHA et al., 2017). Similar behavior was observed with chickpea cultivated under high salinity and nitric oxide application , in which significant increase in shoot and root length was observed (AHMAD et al., 2016), corroborating results obtained with rice (MOSTOFA et al., 2015). However, with the increase in salinity, reduction in the number of germinated seeds was observed, which may be related to the presence of toxic substances, such as malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), which culminated in metabolic and physiological damage to plants, decreasing growth and vigor (DU et al., 2015), and increasing the percentage of abnormal seedlings (data not presented).

Regarding root length (Table 1) of seedlings originated from seeds treated with water (control), G1genotype stands out with the longest root length. However, with treatment with the NO donor at concentration of 1.5 mg L^{-1}, higher results were observed for G3, G4, G5, G6, G7, G8, and G9 genotypes. When increasing the Tadalafil concentration to 2.5 mg L^{-1}, G8 genotype stands out with the highest radicle length. At concentration of 5 mg L^{-1}, seedlings from G7 and G9 genotypes presented the highest root length values, evidencing the action of nitric oxide in stimulating the germination, elongation, and formation of adventitious roots (SCHLICHT et al., 2013; BAI et al., 2014).

Similar behavior wasobserved in the total dry matter of seedlings. However, at dose of 1.5 mg L^{-1}, the genotype that presented the highest total dry matter value was G5 (0.047 mg). At concentration of 2.5 mg L^{-1}, the highest total dry matter values were observed for G3, G6, and G7 genotypes, with 0.030, 0.030, and 0.025 mg, respectively, evidencing the effect of the nitric oxide donor in increasing the germination percentage as a function of the increase in the action of antioxidant enzymes, such as peroxidase, superoxide dismutase, and catalase, which minimize the oxidative stress caused by salinity, being able to correct the nutritional imbalance and influence plant growth. Nitric ox-

ide has a messaging function in response to stress, becoming the product of its interaction with phytohormones (FAN et al., 2014; DU et al., 2015; SANZ et al., 2015).

G2 genotype constituted a distinct group comparing to the other genotypes (Figure 7A). This occurs because its seeds, when under salt stress and without the use of the NO donor, presented 53% of germination and 0.620 of germination speed index, statistically differing from the other genotypes (Table 1).

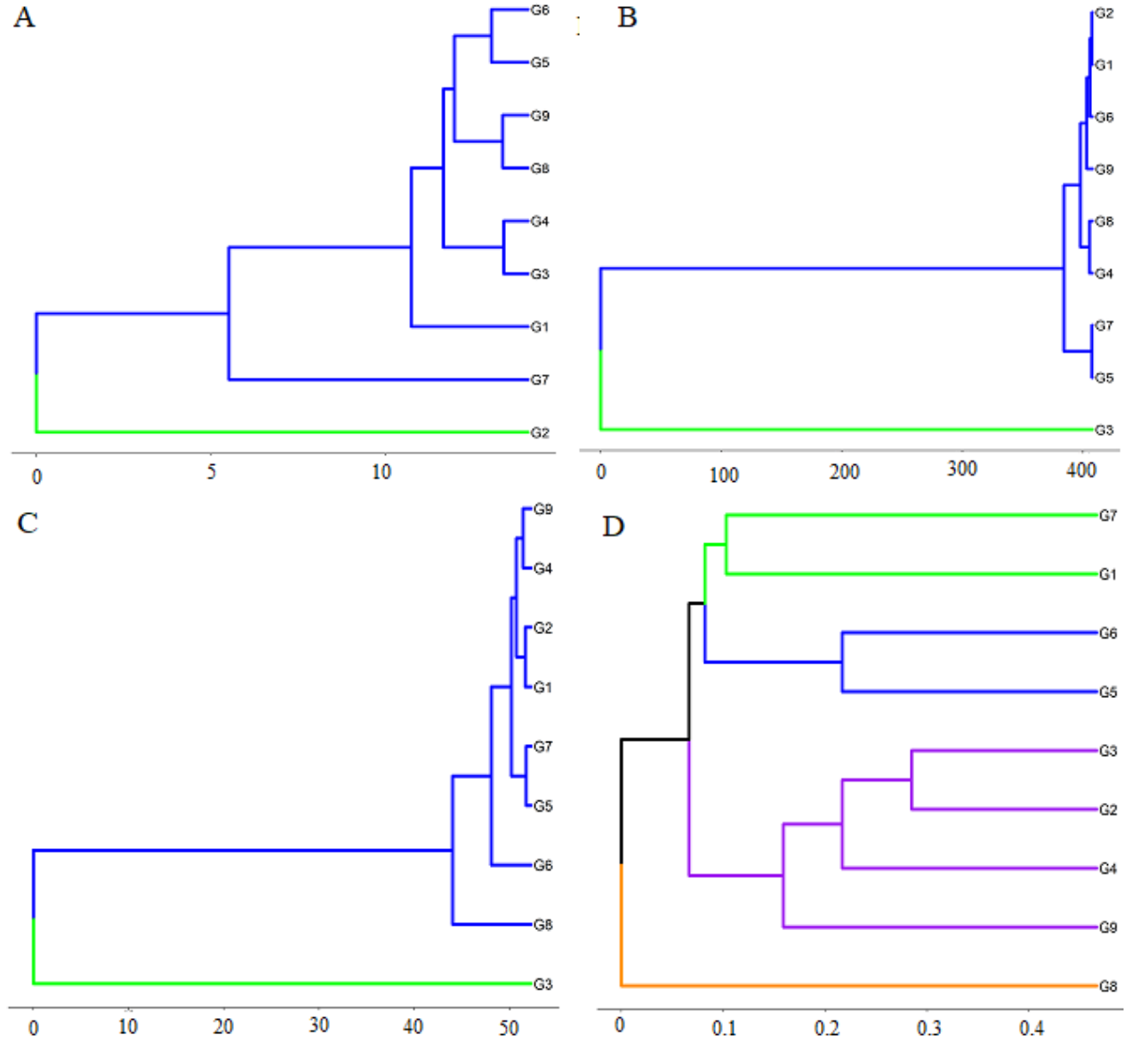

Figure 7 - Representative dendrogram of morphologic [concentrations of 0 mg L^{-1} (A), 1.5 mg L^{-1} (B), 2.5 mg L^{-1} (C)] and genetic dissimilarity based on ISSR markers (D) among the nine *Passiflora mucronata* genotypes, resulting from the analysis of conglomeration obtained by the method of the mean distance between genotypes, using Mahalanobis as measures of genetic distance. Pre-conditioned with different Tadalafil concentrations under salt stress induced with NaCl at -1.2 MPa.

In the dendrogram (Figure 7B), groups were modified: G3 genotype differed from the others, evidencing that with Tadalafil application at dose of 1.5 mg L^{-1} (Table 1), germination increased from 9 to 64%, germination speed index to 1.192, and the mean germination time reduced from 20 to 15 days.

G3 genotype (Figure 7C) remained in a distinct group, compared to the other genotypes. When analyzing treatment with

Tadalafil dose of 2.5 mg L^{-1} (Table 1), the germination rate was 56%, the germination speed index was 1.052, the mean germination time was 14 days, and the total dry matter was 0.030 mg, differing from the other genotypes.

The dendrogram (Figure 7) presents the nine genotypes analyzed by ISSR molecular markers, clustering them into four groups (I: G1 and G7; II: G5 and G6; III: G2, G3, G4, and G9; IV: G8).

G2 and G3 genotypes (Figure 7D) presented genetic similarity. However, when seeds were exposed to salt stress and pre-conditioned with the NO donor, they presented different phenotypic responses and were represented in different groups in dendrograms (Figures 7A, B, and C), differing from G5 and G6 genotypes, which were genetically closer and presented similar phenotypic responses, representing the same phenotypic groups.

The genetic diversity of these of *P. mucronata* genotypes was studied by França et al. (2018), who found different rooting and growth responses among the nine genotypes and suggested that it was caused by the exogenous auxin induction, a behavior that evidenced the existence of intraspecific divergence among *P. mucronata* populations.

Conclusions

The nitric oxide donor, Tadalafil, at concentrations of 1.5 and 2.5 mg L^{-1}, increased the germination speed, shoot length, and total dry mass of seedlings.

The pre-conditioning of *Passiflora mucronata* seeds with the nitric oxide donor Tadalafil at concentrations of 5.0, 7.5, 10.0, and 12.5 mg L^{-1}, determines the death of seeds.

Seeds from G3 genotype, pre-conditioned with Tadalafil at concentrations of 1.5 and 2.5 mg L^{-1}, presented higher germination, germination speed index, mean germination time, shoot length, root length and total dry matter values.

However, under salinity and with the application of the NO donor, seeds presented distinct phenotypic responses, providing intraspecific divergence.

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