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ORIGINAL ARTICLE

Salicylic acid induces acclimation to water deficit in *Phaseolus lunatus* genotypes[1]

Ácido salicílico induz aclimatação ao déficit hídrico em genótipos de *Phaseolus lunatus*

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HIGHLIGHTS:

O primeiro highlight: 'Cara Larga' demonstrated the most response to salicylic acid (SA) and water stress among the three genotypes.
Elicitation with salicylic acid (SA) influenced photosynthetic in all genotypes under stress conditions.
Under conditions of greater water restriction, the application of SA stimulated proline accumulation in the three genotypes.

ABSTRACT: In Brazil, the lima bean is the second most economically significant legume within the genus *Phaseolus*. Climate change, particularly water scarcity, threatens the production of this species. The application of salicylic acid has mitigated the adverse effects of stress. This study aimed to assess the impact of salicylic acid on acclimatisation to water restriction in three genotypes of *Phaseolus lunatus* ('Cara Larga', 'Cearense', and 'Orelha de Vó'). A completely randomised design with a triple factorial included three broad bean genotypes, two pre-conditionings with 1.0 mM salicylic acid and without this elicitor (0.0 mM), and three levels of water availability (75, 50, and 25%), totalling 18 treatments with eight replicates. Physiological and biochemical responses were evaluated after 60 days of treatment. The responses varied among the genotypes. 'Cara Larga' stood out regarding osmoregulatory and antioxidant parameters compared to the other genotypes. In contrast, 'Cearense' showed an increase only in carbohydrates and carotenoids concentrations, while 'Orelha de Vó' exhibited more efficient water use and higher levels of proline under greater water restriction, concurrently with a decline in other parameters. Overall, the 'Cara Larga' genotype appears to be the most responsive to the modulating effects induced by acid application, especially under a water restriction of 25%. Applying, applying salicylic acid under conditions of low water availability may be a strategy for modulating the synthesis of osmoregulatory and antioxidant responses in *P. lunatus*.

Key words: Elicitor, Oxidative stress, Lima bean, Semiarid, Gas exchange

RESUMO: No Brasil, o feijão-fava é a segunda leguminosa mais significativa economicamente dentro do gênero *Phaseolus*. As alterações climáticas, especialmente a escassez de água, ameaçam a produção desta espécie. Neste contexto a aplicação de ácido salicílico mitigou os efeitos adversos do estresse. Este estudo teve como objetivo avaliar o impacto do ácido salicílico na aclimatação à restrição hídrica em três genótipos de *Phaseolus lunatus* ('Cara Larga', 'Cearense' e 'Orelha de Vó'). O delineamento inteiramente casualizado com fatorial triplo incluiu três genótipos de fava, dois pré-condicionamentos com ácido salicílico 1,0 mM e sem esse elicitor (0,0 mM) e três níveis de disponibilidade hídrica (75, 50 e 25%), totalizando 18 tratamentos com oito repetições. As respostas fisiológicas e bioquímicas foram avaliadas após 60 dias de tratamento. As respostas variaram entre os genótipos. 'Cara Larga' destacou-se nos parâmetros osmorregulatórios e antioxidantes em relação aos demais genótipos. Em contrapartida, 'Cearense' apresentou aumento apenas nas concentrações de carboidratos e carotenoides, enquanto 'Orelha de Vó' apresentou uso mais eficiente da água e maiores teores de prolina sob maior restrição hídrica, concomitantemente com declínio em outros parâmetros. No geral, o genótipo 'Cara Larga' parece ser o mais responsivo aos efeitos moduladores induzidos pela aplicação de ácido, especialmente sob restrição hídrica de 25%. A aplicação, a aplicação de ácido salicílico em condições de baixa disponibilidade hídrica pode ser uma estratégia para modular a síntese de respostas osmorreguladoras e antioxidantes em *P. lunatus*.

Palavras-chave: Elicitor, Estresse oxidativo, Fava, Semiárido, Trocas gasosas

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Introduction

Lima bean (*Phaseolus lunatus* L., Fabaceae) is economically and socially significant in Brazil and is cultivated predominantly in the semi-arid regions of the northeast. These regions are characterised by irregular rainfall, shallow soils, and high temperatures, representing an additional challenge owing to high evapotranspiration (Marçal et al., 2019).

Water deficits cause losses in agricultural production and threaten sustainable agriculture (Rosa et al., 2020). Due to climate change, an increase in dry areas and irregular rainfall is expected, thereby decreasing agricultural production and increasing unproductive areas. Water stress is critical for plants because it induces changes in their morphophysiology and biochemistry, negatively affecting their growth, development, and productivity (Zoghi et al., 2019).

Understanding and developing tools to increase plant drought tolerance is critical in the face of rising global temperatures and more extended periods of drought (Dawood et al., 2021). However, the susceptibility of plants to drought stress varies with the intensity, duration, and stage of development (Zoghi et al., 2019). To cope with these conditions, osmoprotective substances, growth regulators, and signalling molecules have been used to promote tolerance to biotic and abiotic stresses (Khan et al., 2022).

Salicylic acid (SA) is a phenolic compound with multiple functions in plants. It regulates the physiological processes of plant growth and development, such as photosynthesis, respiration, flowering, and stomatal conductance (Li et al., 2019). SA is associated with signalling and stress resilience and induces defence mechanisms (Debona & Rodrigues, 2018; Li et al., 2019).

Based on the results mentioned above, this experimental study assessed the impact of salicylic acid on physiological and biochemical responses in three *P. lunatus* genotypes subjected to three levels of water availability to mitigate the effects of water deficit.

Material and Methods

Lima bean genotypes from the Instituto Agronômico de Pernambuco (IPA) were evaluated, namely: 'Cara Larga', 'Cearense' (with a cultivation cycle of up to 90 days) and 'Orelha de Vó' (with a cycle of ~115 days). These genotypes exhibit indeterminate growth and satisfactory agronomic and commercial characteristics. They are characterised as landrace seeds passed on from generation to generation by family farmers.

The experiment was conducted in the greenhouse of the Federal Rural University of Pernambuco (UFRPE), located at the geographical coordinates 8° 01' 8.0" S and 34° 56' 44.0" W, at an approximate altitude of 10 meters a.s.l. This facility falls under the purview of the Department of Plant Science, and observations were conducted from August to December 2019.

Pots with a capacity of 5.5 dm^3, containing a compound of washed sand, soil, and coconut fibre (3:2:1, v/v), were used. Three seeds of each genotype were sown. After 15 d, thinning was performed, leaving only one plant per pot (experimental unit).

Thirty days after emergence (DAE), 100 mL of 1.0 mM salicylic acid (SA) solution was sprayed onto the shoots of each plant, except for the control plants. Nine days after the SA application, treatments with different water availabilities were started: 25, 50, and 75% of pot capacity (75% was considered the control). The experimental design was completely randomised, with a triple factorial scheme: three lima bean genotypes × two preconditioning with 1.0 mM SA and without elicitor (0.0 mM)) × three water availabilities (WA: 75, 50, and 25%), totalling 18 treatments with eight replicates.

The pot capacity (PC) was defined as the water content retained by the soil until drainage ceased, according to Souza et al. (2000). Irrigation was performed on alternate days, and the average soil moisture of the six plants per treatment was measured using a soil moisture device (Hydrosense II), allowing for the necessary watering to maintain the established treatments. At the end of the experimental period (60 days), growth, gas exchange, and biochemical (primary metabolism and antioxidant) analyses were performed.

The stem length (SL) was evaluated using a tape measure. In addition, materials were collected to evaluate shoot and root dry weights.

Gas exchange analysis was performed on healthy and fully expanded leaves in the middle third of the plant between 11:00 am and 2:00 pm. Stomatal conductance (g_s - mol H_2O m^{-2} s^{-1}), transpiration rate (E - mmol H_2O m^{-2} s^{-1}), net photosynthesis (A - µmol CO_2 m^{-2} s^{-1}) and leaf temperature (LT - ºC) were measured using an infrared gas analyser (IRGA-LI 6400XT, LI-COR, USA). The CO_2 concentration and ambient luminosity readings were taken, with PARin = 562.91 µmol m^{-2} s^{-1}.

Healthy and fully expanded leaves from the middle third of the plant were collected and frozen in liquid nitrogen for subsequent biochemical analyses. All readings were obtained using a UV-Visible spectrophotometre (Bel Photonics).

The leaves (200 mg) were macerated and filtered, and the extract was prepared with 20 mL of ethanol (80%). The soluble carbohydrate content was measured using the anthrone method Bezerra & Barreto (2011) described. Absorbance was measured using a spectrophotometre at 620 nm. The content is expressed as mg g^{-1} fresh weight (FW).

To determine proline content, 200 mg of leaves were macerated in liquid nitrogen until homogenisation to prepare the extract using 5 mL of a 3% solution of sulfosalicylic acid as a solvent. The absorbance was read at 520 nm using a spectrophotometre based on the methodology described by Bates et al. (1973) and modified by Bezerra & Barreto (2011). The content was expressed in µmol g^{-1} of FW.

Chlorophyll *a* and *b* (total chlorophyll) and carotenoid contents were analysed using 100 mg fresh weight macerated in 80% (v/v) acetone. The extract was then filtered through a fine-mesh nylon screen. Readings to determine the concentrations of chlorophyll *a, b* and carotenoids were taken at 663, 645, and 470 nm, respectively, according to Lichtenthaler & Buschmann (2001), with adaptations from Bezerra & Barreto (2011). The content was expressed in g kg^{-1} FW.

Two hundred milligrams of leaves (200 mg) were macerated with 40 mg of polyvinylpolypyrrolidone (PVPP) in liquid nitrogen, followed by homogenisation in 2 mL of

100 mM potassium phosphate buffer (pH 7.5) containing 3 mM 1,4-dithiothreitol (DTT) and 1 mM ethylenediamine tetraacetic acid (EDTA). The resulting extract was then centrifuged at 10,000 rpm at 4 °C for 20 minutes, and the supernatant was utilised to analyse total protein content, following the method described by Bradford (1976). The content was expressed in mg g^{-1} FW.

Superoxide dismutase (SOD) activity was determined according to the method described by Giannopolitis & Ries (1977) with modifications. The reaction was composed of 85 mM sodium phosphate buffer, pH 7.8 (1.765 mL), methionine (780 µL), blue nitro tetrazolium (225 µL), EDTA (30 µL), riboflavin (150 µL) and protein extract (50 µL). The tubes were irradiated with fluorescent light (15 W) for 5 min. Absorbance was measured at 560 nm using a spectrophotometre. For calculation, the percentage of inhibition obtained, the sample volume and the protein concentration in the sample (µg μL^{-1}) were adopted. Enzymatic activity is expressed as SOD units in mg^{-1} of protein.

Catalase (CAT) activity was determined using the method described by Havir & McHale (1987) with modifications by Azevedo et al. (1998). The reaction containing 100 mM potassium phosphate buffer, pH 7.0 (1,390 µL), 0.5 M hydrogen peroxide (60 µL) and protein extract (50 µL) at 25 °C was monitored for 60 seconds under 240 nm wave in spectrophotometre. Enzymatic activity was expressed in µmol H_2O_2 mg^{-1} protein min^{-1}.

The ascorbate peroxidase (APX) activity was determined as described by Nakano & Asada (1981). The reaction medium consisted of 650 µL of 80 mM potassium phosphate buffer (pH 7.5), 100 µL of 5 mM ascorbate, 100 µL of 1 M EDTA, 100 µL of 1 mM H_2O_2, and 50 µL of the extract protein. APX activity was determined by monitoring the rate of ascorbate oxidation at 290 nm in a spectrophotometre at 30 °C for 60 seconds. Enzymatic activity was expressed in mg^{-1} protein min^{-1}.

The extract was prepared with 200 mg of macerated plant material in 2 mL of 0.1% trichloroacetic acid (TCA) with the addition of 20% PVPP and centrifuged for 5 min at 10,000 rpm to determine malondialdehyde (MDA) content. An aliquot (250 µL) was mixed with 1 mL of TCA (20%) and TBA (0.5% thiobarbituric acid) solution. The solution was heated at 95°C for 30 minutes, cooled for 10 minutes, and centrifuged for 10 minutes at 10,000 rpm. The absorbance was measured using a spectrophotometre at 535 nm with a residue at 600 nm (Heath & Packer, 1968). The content was expressed in µmol g^{-1} of FW.

Hydrogen peroxide (H_2O_2) content was determined using an extract prepared for MDA. The reaction consisted of the supernatant (200 µL), potassium iodide (800 µL), and 100 mM phosphate buffer (200 µL, pH 7.5). The solution was then left on ice for an hour in the dark. Subsequently, the absorbance was read using a spectrophotometre at 390 nm (Alexieva et al., 2001). The content was expressed in µmol g^{-1} of FW.

Analysis of variance was performed. The normality of the residuals was assessed using the Shapiro-Wilk test, and logarithmic and square root transformations were applied if not met. Mean comparisons were conducted using the Tukey test ($p \leq 0.05$), using the R software, version 4.3.2, package ExpDes.pt (Ferreira, et al. 2014).

Results and Discussion

After 60 days of the experiment, the interplay among genotypes, salicylic acid (SA), and varying water availability (WA) substantially influenced biochemical and physiological variables while exhibiting a limited impact on biometric measures. Only root length and shoot dry weight significantly differed in the latter group.

Root length was greater in the most hydrated treatment (WA 75%) with SA (Figure 1B) for the 'Cara Larga' and 'Orelha de Vó' genotypes. On the other hand, under greater water restriction (WA 25%) with SA, the 'Cearense' genotype exhibited an increase in root length compared to the 'Orelha de Vó' genotype (Figure 1B). Concerning the shoot dry weight, the genotypes 'Cearense' and 'Orelha de Vó' exhibited similar behaviour in the more hydrated treatments (WA 75%) without the application of SA and with WA 50% with SA (Figure 1C).

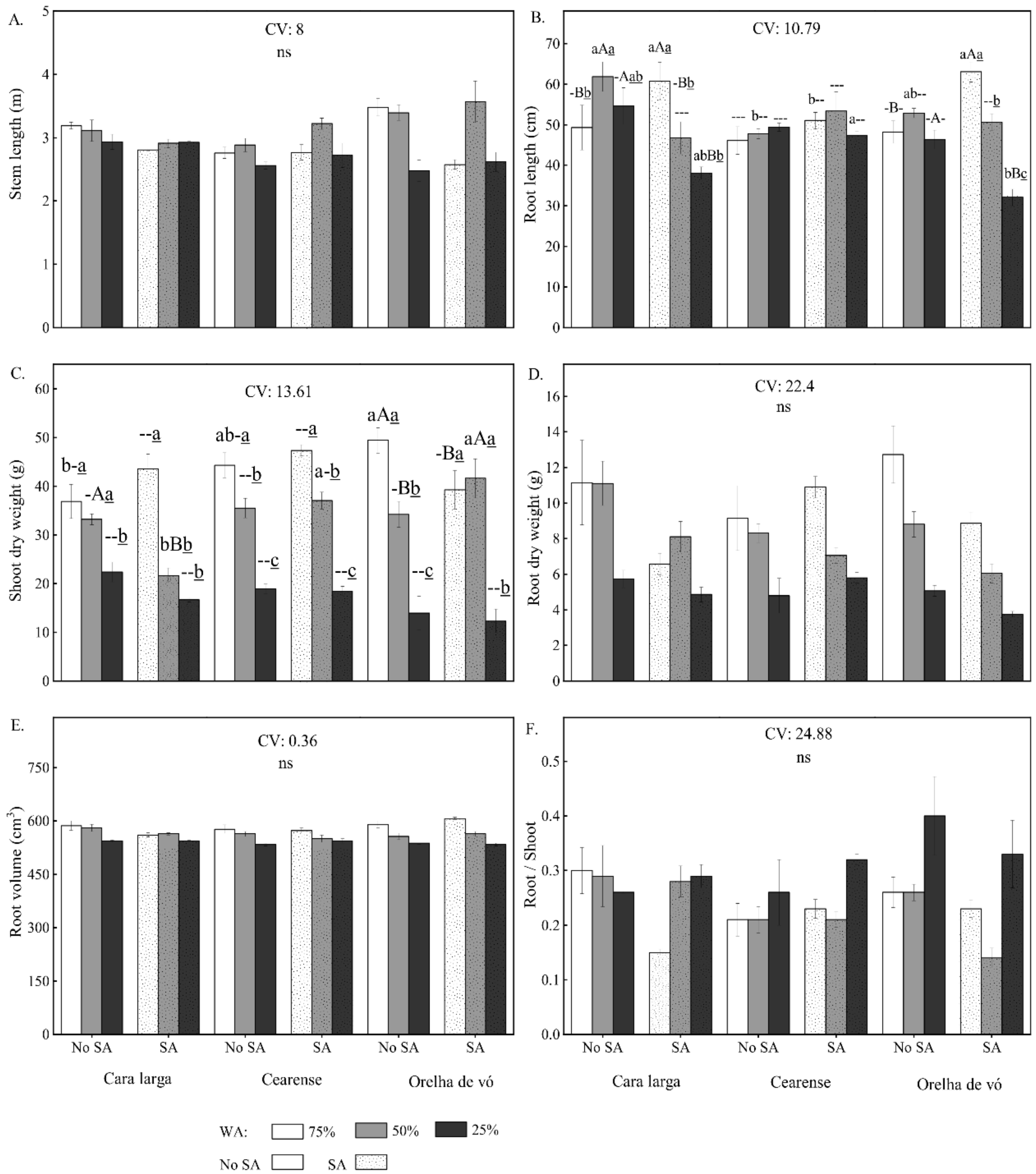
The increased shoot dry weight and root length in 'Orelha de Vó' due to SA application, since it participates in numerous regulatory functions in plant metabolism, being able to activate defence mechanisms against environmental stresses, and more broadly, improvements in growth and accumulation of dry biomass in the presence of SA, and this can be explained by the association of this elicitor with other growth regulators, which induces greater tolerance to water stress (Li et al., 2019).

Some studies have demonstrated the effectiveness of SA (Lima et al., 2019), where the application of SA to common bean (*P. vulgaris*) increased plant dry mass, root length, and germination. These results could be observed in plants of the 'Cearense' genotype, which increased the shoot dry weight and root length when treated with SA under low water availability.

Therefore, based on the results, all genotypes were influenced as water restriction intensified, affecting shoot growth and demonstrating sensitivity mainly to the treatment with lower water availability.

Among the physiological variables, *A*, *gs*, and *E* demonstrated heightened responsiveness to SA and water availability. However, each genotype exhibited a distinctive behaviour. In the 'Cara Larga' genotype, *A*, *gs* and *E* exhibited greater responsiveness in the WA 50% treatment with SA, showing similar behaviour to the 75% treatments with and without SA (Figures 2A-C). However, the presence of SA highlighted the *WUE* in 'Cara Larga' in the WA 25% treatment, 'Cearense' in WA 75 and 50% and 'Orelha de Vó' in WA 25%. (Figure 2D). These variables *A*, *gs*, and *E* were similarly affected in 'Cearense' in WA by 75% with SA (Figure 2A-D).

In 'Orelha de Vó', the WA 50% treatment highlighted *gs* in the absence of SA and *E* with SA. In WA 25% with SA, a higher WUE was observed (Figure 2D), distinguishing it from the other genotypes. Furthermore, the presence of SA contributed to higher rates of A, gs, and E at WA 50% in all three *P. lunatus* genotypes (Figures 2A, B, and C). The use of SA at adequate concentrations can increase photosynthetic capacity, and the response of plants to this hormone depends on the environmental conditions, species, variety, genotype, concentration, form, and time of application (Batista et al., 2019). It is worth noting that the closure of stomata reduces transpiration rates, causing an increase in leaf temperature



The significance is composed of three levels, considering the triple factorial: The first level (lowercase letters) indicates differences between genotypes, the second (uppercase letters) indicates differences resulting from the application of salicylic acid (SA), and the third level (underlined lowercase letters) indicates variations related to water availability (WA) by the Tukey test at p ≤ 0.05. The dashed line (-) indicates that the interaction between these factors was insignificant, and 'ns' indicates a non-significant interaction between genotypes

Figure 1. Stem length (A), root length (B), shoot dry weight (C), root dry weight (D), root volume (E), and root/shoot ratio (F) of *Phaseolus lunatus* genotypes subjected to the absence and presence of salicylic acid (SA) (0 and 1.0 mM) and cultivated under different water availability conditions (75, 50, and 25% water availability (WA) at 60 days

and affecting the functioning of photosystems and carbon assimilation (Batista et al., 2019). However, this was not observed in the present study.

However, SA improved photosynthetic parameters in 'Cara Larga' under WA by 50% compared to other genotypes without affecting leaf temperature, indicating the applicability of SA to mitigate the effects of reduced water availability on photosynthesis. The reduction in soil water availability causes reductions in stomatal conductance, directly affecting transpiration, photosynthesis, and leaf temperature, which may harm agricultural production during severe droughts (Rosa et al., 2020). Even with an increase in gs in 'Cara Larga' due to

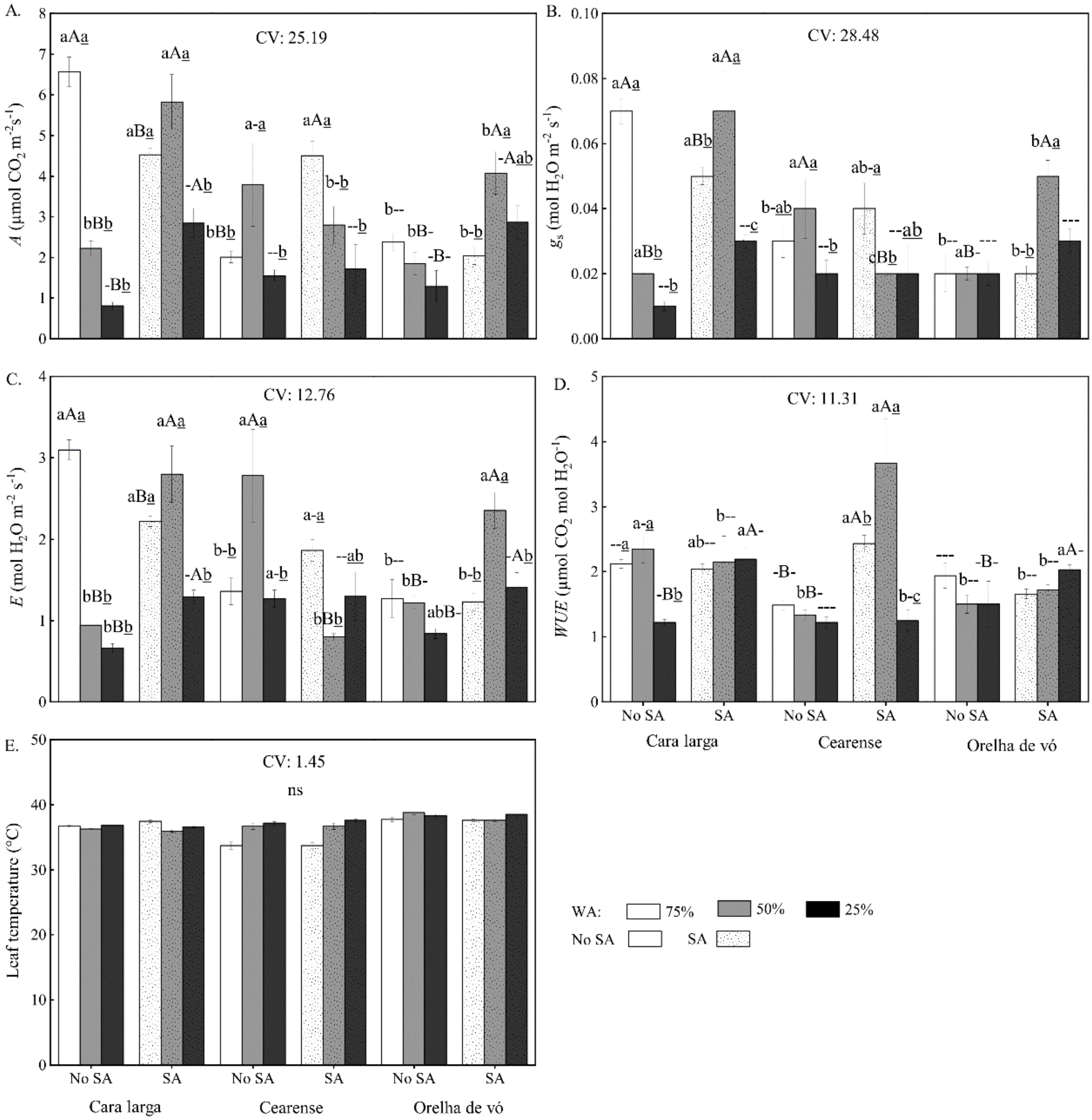


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Figure 2. Net photosynthesis (A- A), stomatal conductance (g_s- B), transpiration (E- C), water use efficiency (WUE- D), leaf temperature (E) of *Phaseolus lunatus* subjected to the absence and presence of salicylic acid (SA) (0 and 1.0 mM) and cultivated under different water availability conditions (75, 50, and 25% water availability (WA) at 60 days

water restriction of 50%, the increase in the WUE found in SA treatments in WA 25% for 'Cara Larga' and 'Orelha de Vó', and in WA 50% in 'Cearense' suggests that the use of SA optimised the efficient use of water in the genotypes evaluated here.

The use of SA reiterates that under normal conditions, net photosynthesis (A) does not depend exclusively on chlorophyll but also on other factors, such as Rubisco activity, g_s and carbon uptake (Batista et al., 2019). The indirect effects of high temperature highly influence net photosynthesis, the high vapour pressure deficit and the consequent decrease in stomatal conductance (Li et al., 2019).

Despite numerous physiological modulations, the most pronounced responses were observed in osmoregulators, such as proline, total soluble carbohydrates, and carotenoids. Among the genotypes, 'Cara Larga' had the highest proline content in WA, 75 and 50% without SA and in WA, 50% with SA. 'Cearense' modulated in WA 25% with and without SA, and 'Orelha de Vó' in WA 25 and 75% with SA. SA led to proline accumulation in the treatment with more significant water restriction (WA 25%) in 'Cara Larga', 'Cearense' and 'Orelha de Vó' (Figure 3A).

Furthermore, 'Cearense' showed higher levels of total soluble carbohydrates (Figure 3C), total chlorophyll (Figure

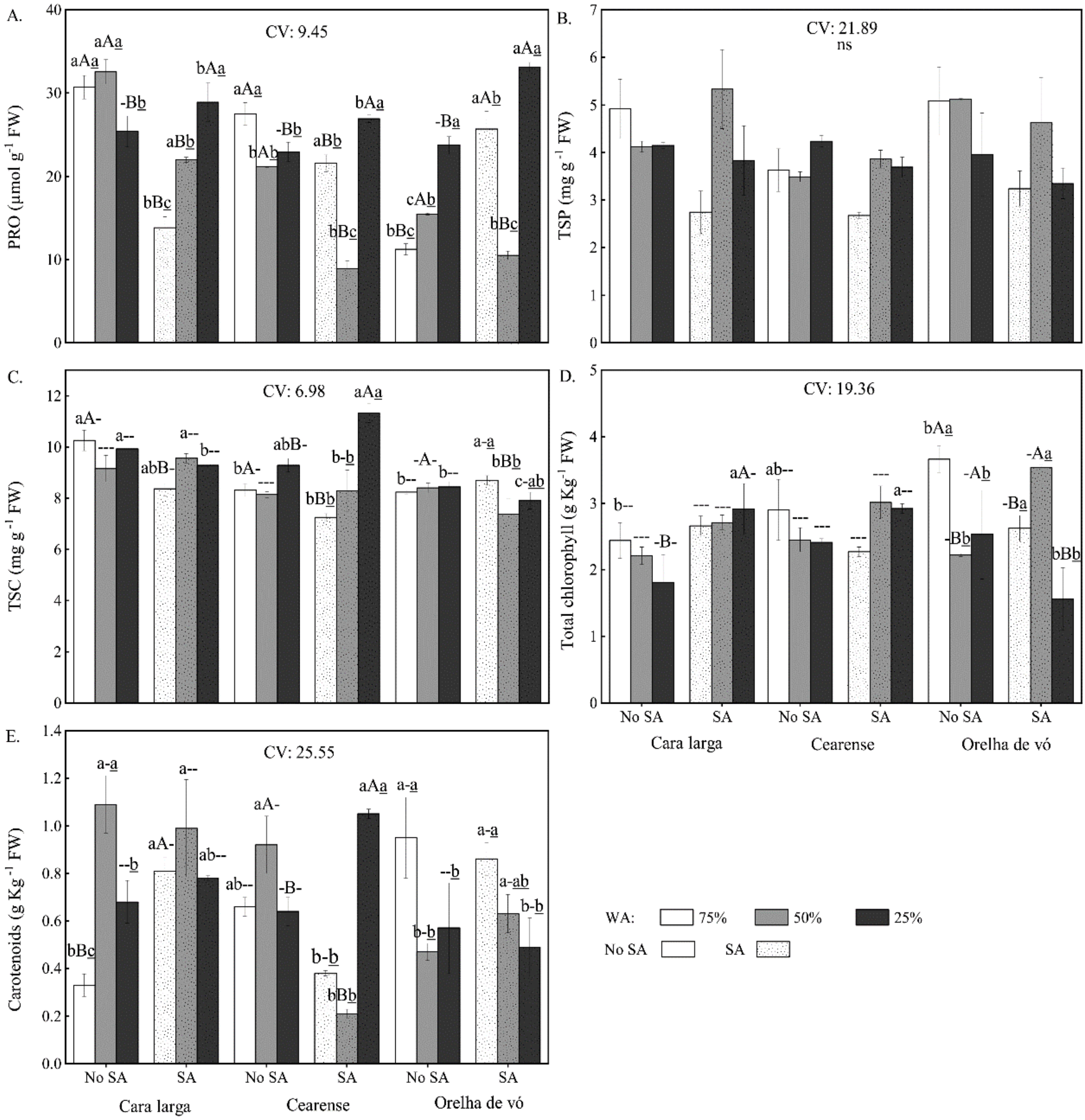

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Figure 3. Proline (PRO- A), total soluble proteins (TSP- B),total soluble carbohydrates (TSC- C), total chlorophyll (D), carotenoids (E) of *Phaseolus lunatus* genotypes subjected to the absence and presence of salicylic acid (SA) (0 and 1.0 mM) and cultivated under different water availability conditions (75, 50, and 25% water availability (WA) at 60 days

3D), and carotenoids (Figure 3E) in WA 25% with SA. SA increased total chlorophyll contents in WA by 25% in 'Cara Larga', similarly observed in 'Orelha de Vó' in WA by 50%. The accumulation of osmoregulators such as soluble carbohydrates and carotenoids was enhanced under the most water restriction (WA 25%), especially in the presence of SA in 'Cearense' (Figures 3B and E).

The ability to maintain adequate levels of chlorophyll under unfavourable conditions may be related to tolerance and photosynthetic efficiency, and such feedback may be linked to the activity of enzymes that participate in chlorophyll biosynthesis or even to mechanisms that reduce the degradation of these pigments by acting as antioxidant agents (Dumanović et al., 2021). Additionally, proline is vital for plant responses to abiotic stresses because it is an osmoregulator produced in response to stress (Dikilitas et al., 2020). Despite the emphasis on the increase in proline in 'Cara Larga' under WA 50%, the results presented here suggest that the use of foliar spraying with SA induced an increase in proline production in treatments with greater water restriction, providing an improvement in osmotic protection for *P. lunatus* genotypes.

In the context of oxidative stress and antioxidant enzymes, in the WA 25% SA treatment, the 'Cara Larga' and 'Cearense' genotypes exhibited higher superoxide dismutase (SOD) activity compared to 'Orelha de Vó' (Figure 4A). The presence of SA only caused an increase in APX in 'Orelha de Vó' at WA 50% (Figure 4C). The presence of SA decreased CAT and APX activity in the other genotypes and caused accumulation of hydrogen peroxide (H_2O_2) in 'Cearense' and 'Orelha de Vó' in the WA 50 and 25% treatments, respectively (Figures 4E).

However, among the three genotypes, only 'Cearense' demonstrated an accumulation of malondialdehyde (MDA, Figure 4D). In the WA 50% + SA treatment, the 'Cara Larga' genotype accumulated H_2O_2 (Figure 4E) and a reduction in enzymatic activity. The 'Cearense' genotype stood out for MDA (Figure 4D) accumulation, while 'Orelha de Vó' exhibited higher SOD (Figure 4A) and ascorbate peroxidase (APX) activity (Figure 4C). Regarding antioxidant responses in the WA 25% + SA treatment, 'Cara Larga' displayed higher SOD (Figure 4A) activity and MDA (Figure 4D) and H_2O_2 (Figure

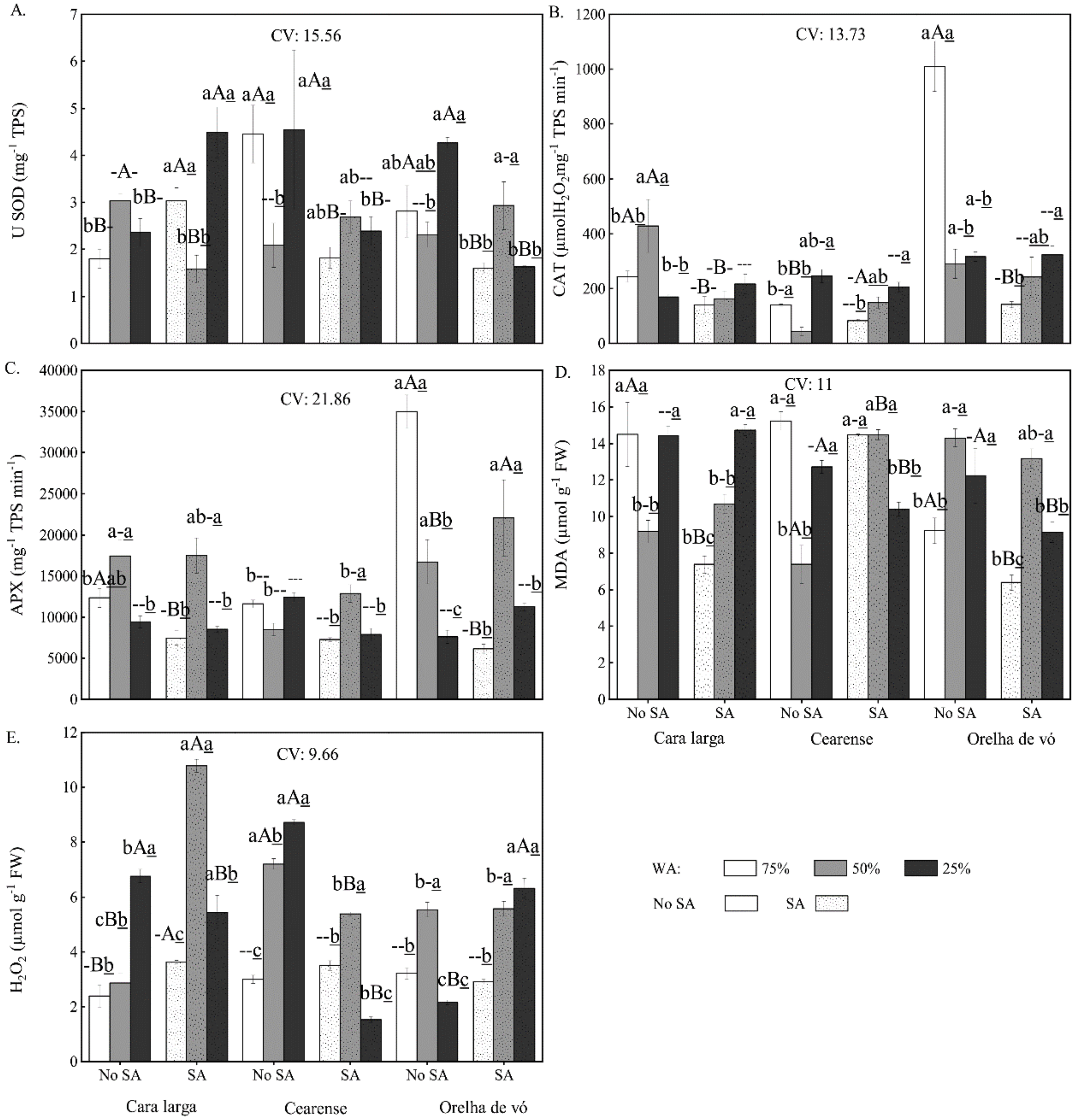

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Figure 4. Superoxide dismutase (SOD- A), catalase (CAT- B), ascorbate peroxidase (APX- C), malondialdehyde (MDA- D), and hydrogen peroxide (H_2O_2- E) of *Phaseolus lunatus* genotypes subjected to the absence and presence of salicylic acid (SA) (0 and 1.0 mM) and cultivated under different water availability conditions (75, 50%, and 25% water availability (WA) at 60 days

4E) accumulation, whereas 'Orelha de Vó' exhibited a similar behaviour to H_2O_2.

The presence of SA increased SOD activity in 'Cara Larga' in the WA 75 and 25% treatment, catalase (CAT) activity in 'Cearense' in the WA 50% treatment, and APX activity in 'Orelha de Vó' in the WA 50% treatment. MDA accumulation was reduced in all SA treatments, irrespective of genotype, even with H_2O_2 accumulation in 'Cara Larga' in the WA 50 and 25% treatments and in 'Orelha de Vó' in the WA 25% treatment.

Among the different water treatments, the presence of SA in 'Cara Larga' resulted in higher SOD activity in treatments with both higher and lower hydration levels (WA 25 and 75%), along with increases in APX and H_2O_2 in the WA 50% treatment and MDA in the WA 25% treatment. In 'Cearense', SA increased CAT and APX activity in the WA 25 and 50% treatment, respectively, with the accumulation of MDA and H_2O_2 in WA 50%. The same pattern was observed in 'Orelha de Vó'; however, there was an increase in SOD activity in the WA 50% treatment, and in both the WA 50 and 25% treatments, there was higher CAT activity in both genotypes, with H_2O_2 accumulation in 'Orelha de Vó' and MDA in WA 50%.

The effects of SA as a mitigating agent of water stress include increases in the antioxidant capacity of plants and the constancy of membranes due to the decrease in the level of lipid peroxidation, increase in photosynthetic capacity, and accumulation of biomass (Khan et al., 2022). In addition, SA is essential for regulating several physiological processes in plants and is a crucial component of the translation signals of the main pathways that refer to systemic resistance present (Debona & Rodrigues, 2018). One of the consequences of water deficit is an imbalance in the intracellular redox state, which causes excessive production of ROS, among which H_2O_2 stands out. ROS can cross membranes and diffuse between compartments, causing membrane damage through lipid peroxidation (Tian et al., 2016).

Salicylic acid has been shown to minimise the deleterious effects on plants under stressful conditions (Khan et al., 2022). Batista et al. (2019) found that SA decreased H_2O_2 overproduction in plants subjected to water stress. In the present work, this result was observed in the 'Cearense' and 'Cara Larga' genotypes treated with SA in the treatment with 75% water availability. On the other hand, knowing that H_2O_2 acts in the signalling of stress, the presence of SA also helped in this response, observing the increase of H_2O_2 in the 'Cara Larga' and 'Orelha de Vó' genotypes.

Furthermore, the best acclimatisation response found was in the 'Orelha de Vó' and 'Cearense' genotypes when subjected to lower water availability (25%) in the presence of SA, reducing the production of malondialdehyde (MDA), avoiding damage to cell membranes. According to Torun (2019), exogenous SA can regulate the defence enzymes of the antioxidant system. In this sense, the greater activity of CAT and APX under stress conditions can be explained by the need to eliminate H_2O_2 produced by SOD activity as well as the possible rate of photorespiration (Nasirzadeh et al., 2021).

The high activity of CAT is attributed to plants under adverse conditions, as it is characterised as an alternative means for converting ROS, such as H_2O_2, into water and molecular oxygen, thus avoiding damage to cellular structures under water restriction (Gupta, 2010). This behaviour was observed in the 'Cara Larga' genotype, with an increase in MDA in the condition of lower water availability (25%) in plants treated with SA, but, on the other hand, SOD activity was higher in this same treatment.

In higher concentrations, Carotenoids, which essentially protect the photosynthetic mechanism, indicate genotypes acclimated to water deficit conditions (Dumanović et al., 2021). Under these conditions, where oxidative stress can occur, carotenoids also cooperate in the non-enzymatic antioxidant defence system, decreasing the formation of reactive oxygen species (ROS) (Dumanović et al., 2021). The results show that the 'Cearense' genotype increases total chlorophyll and carotenoid contents in treatments with lower water availability (25%), demonstrating acclimatisation to water deficit in the presence of SA.

The results of this study highlight the crucial role of salicylic acid (SA) in the response of plant genotypes subjected to different levels of water restriction (WA 25, 50, and 75%). Although biometric parameters such as root length and shoot dry weight were minimally affected, physiological responses were notably influenced by the interaction between SA and water availability. Specific genotypes, such as 'Cara Larga', excelled in CO_2 net assimilation rates (*A*), stomatal conductance (*gs*), and transpiration (E), whereas osmoregulators, such as proline and carotenoids, play significant roles in plant adaptation to water restriction.

These findings suggest that SA is vital in modulating plant responses to water stress by affecting various physiological and biochemical parameters. This in-depth understanding of genotype-acid-water interactions is essential for developing effective management strategies to optimise plant performance under water stress conditions, contributing to agricultural sustainability and productivity.

Conclusion

1. The application of salicylic acid indicated significant benefits for the 'Cara Larga', 'Cearense' and 'Orelha de Vó' genotypes, improving photosynthetic efficiency, in addition to osmoregulatory adjustments and reducing oxidative stress in conditions of lower water availability, promoting the mitigation of the adverse effects of water stress.

2. Genotype 'Cara Larga' presented enhanced responses due to salicylic acid application under water restriction, increasing osmoregulators and antioxident enzymes, thus reducing oxidative damage and maintaining photosynthetic activity. Therefore, salicylic has emerged as an alternative for mitigating water stress's physiological and biochemical effects in these *Phaseolus lunatus* genotypes.

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