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Salicylic acid and proline modulate water stress tolerance in a traditional variety of cowpeas[1]

Ácido salicílico e prolina modulam tolerância ao estresse hídrico em variedade tradicional de feijão-caupi

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HIGHLIGHTS:

Application of proline attenuates the deleterious effect of water deficit in cowpea.
The use of salicylic acid reduces antioxidant enzyme activity in cowpea.
Salicylic acid increases protein concentrations in cowpea under water stress.

ABSTRACT: Exogenous applications of chemical compounds stimulate changes in plant metabolism and promote tolerance to different environmental stresses, including water deficit. This study aimed to evaluate the ability of salicylic acid (SA) and proline (PRO) to reduce water stress in a traditional variety of cowpea in a typical Brazilian semiarid climate. A completely randomized design was used in a 2 × 4 factorial scheme, with five replicates. Two irrigation regimes were evaluated corresponding to 100% (W100) and 50% of daily evapotranspiration (W50), respectively, with the addition of the following four attenuators: control (distilled water), SA (550 mg L^{-1}), PRO (690 mg L^{-1}), and 690 mg L^{-1} PRO + 550 mg L^{-1} SA. The treatments promoted changes in osmotic and antioxidant metabolism, which may contribute to the tolerance mechanisms of cowpea plants to water stress. The application of SA increased osmoregulator synthesis and protein concentrations, and modulated antioxidant enzyme activity in the cowpea plants under water stress. PRO concentrations increased synergistically in plants treated with PRO and SA, particularly in 50% of water replacement.

Key words: *Vigna unguiculata* L. (Walp), total free amino acids, antioxidative metabolism, water deficiency

RESUMO: Aplicações exógenas de compostos químicos estimulam modificações no metabolismo vegetal e conferem tolerância a diferentes estresses ambientais, incluindo o déficit hídrico. Esta pesquisa teve como objetivo avaliar os efeitos do uso de ácido salicílico (AS) e prolina na redução do estresse hídrico em uma variedade tradicional de feijão-caupi em um clima típico do semiárido brasileiro. Foi utilizado um delineamento inteiramente casualizado em esquema fatorial 2 × 4 com cinco repetições. Foram utilizadas duas lâminas de irrigação, correspondentes a 100% (W100) e 50% da evapotranspiração diária (W50) e quatro tratamentos com atenuadores: controle (água destilada), AS (550 mg L^{-1}), prolina (690 mg L^{-1}) e 690 mg L^{-1} prolina + 550 mg L^{-1} AS. Os elicitores promoveram alterações no metabolismo osmótico e antioxidante, o que pode contribuir para o mecanismo de tolerância das plantas de feijão-caupi sob restrição hídrica. A aplicação de ácido salicílico aumentou a síntese de osmorreguladores, a concentração de proteínas e modulou a atividade de enzimas antioxidantes em plantas de feijão-caupi sob estresse. A concentração de prolina aumentou sinergicamente nas plantas tratadas com prolina e AS, principalmente em 50% de reposição de água.

Palavras-chave: *Vigna unguiculata* L. (Walp), aminoácidos livres totais, metabolismo antioxidativo, deficiência hídrica

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Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is an important crop in tropical regions of the world, with high social and economic value. However, cowpea productivity is strongly affected by water shortages in semiarid regions, where water deficit occurs at one or multiple stages of plant development (Jayawardhane et al., 2022).

To minimize the effects of water deficit, plants use strategies to maintain internal water content to protect cellular structures and mitigate the deleterious effects of the accumulation of reactive oxygen species (ROS). Osmoregulatory compounds can accumulate in plant tissues, promoting the absorption of water from the soil (Coelho et al., 2018). In addition, antioxidant enzymes enhance the ability of plants to maintain ROS at an adequate level to reduce the damage to cellular structures caused by water stress (El-Taher et al., 2022; Melo et al., 2022a; Santos et al., 2022).

Compounds such as salicylic acid (SA) and proline (PRO) have several beneficial effects in plants, attenuating the detrimental consequences of water deficit. Both SA and PRO promote antioxidant enzyme activity and the non-enzymatic removal of free radicals (Lee et al., 2019; Santos et al., 2022), whereas PRO accumulation can also contribute to osmoregulation. Consequently, these compounds improve plant growth and yield (Merwad et al., 2018). SA also exerts regulatory action on leaf gas exchange (Bekka et al., 2018) and contributes to osmotic adjustment in stressful environments (Dutra et al., 2017; Khan et al., 2019).

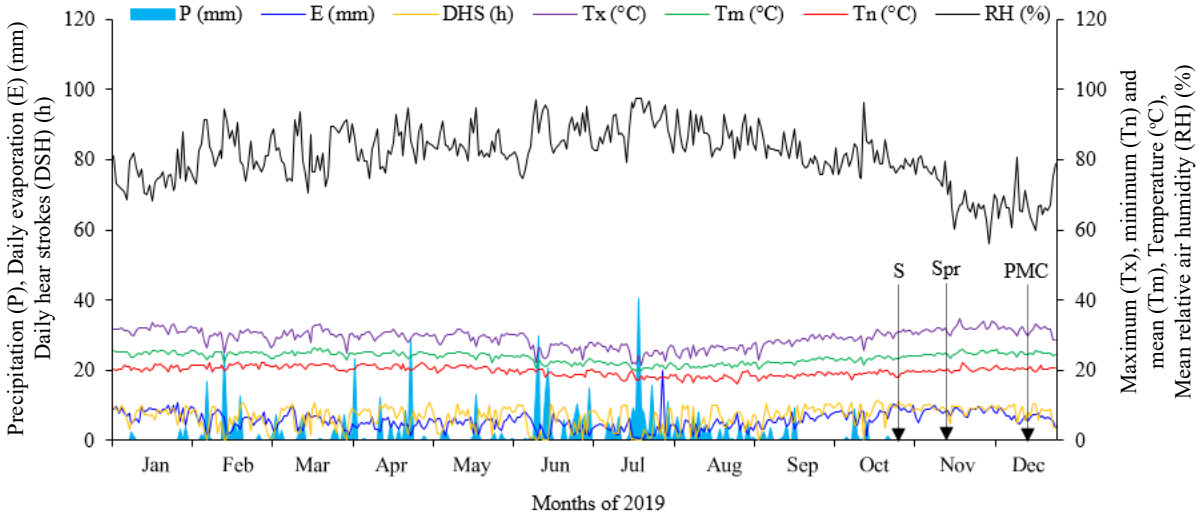
From the perspective of cowpea cultivation in a semiarid environment, it is essential to maintain high yields during periods of water deficit by adopting technologies that improve plant adaptation to water stress. This study aimed to evaluate the ability of SA and PRO to reduce water stress in a traditional variety of cowpea in a typical Brazilian semiarid climate.

Material and Methods

This research was conducted at an experimental site at the State University of Paraíba, Campus I, Campina Grande, Paraíba state, Brazil (07°12'42.99"S, 35°54'36.27"W) between October to December 2019. The experimental site has an altitude of 521 m above sea level, and according to the Köppen classification, the climate of the region is BSw'h', characterized by hot and semi-arid conditions with distinct rainy (February to July) and dry (August to January) seasons. The annual average precipitation is 850 mm, and the average temperature is 27 °C. During the experimental period, meteorological variables (precipitation, P; mean air temperature, Tm; minimum air temperature, Tn; maximum air temperature, Tx; mean relative humidity of the air, RH; daily evaporation, E; daily heat stroke obtained by radiometry, DHS) were monitored by an automatic weather station (7°13'32.0" S, 35°54'17.0" W, altitude = 546.27 m asl, Figure 1).

Water replacement (W), SA, and PRO concentrations were evaluated. Water replacement was either 100% (W100) or 50% of daily evapotranspiration (W50). SA and PRO were used separately and in a combination alongside a control treatment (without application), at 550 mg L^{-1} SA (El-Areiny et al., 2019), 690 mg L^{-1} PRO (Merwad et al., 2018), and 550 mg L^{-1} SA + 690 mg L^{-1} PRO. These combinations resulted in eight treatments organized in a completely randomized design in a 2 × 4 factorial scheme with five replicates. Each replicate consisted of two plants per 20 L pot.

Cowpea, variety Paulistão seeds were obtained from the municipality of Pombal (Paraíba, Brazil). Seeds were classified to eliminate those with damage and/or malformation. After classification, the seeds were treated with Captan® fungicide at a rate of 0.22 g $100g^{-1}$ of seeds for 24 hours. Sowing was carried out manually in polyethylene pots (20 L) filled with Entisol with the following attributes: sand = 659 g kg^{-1}, silt = 101 g kg^{-1}, clay = 240 g kg^{-1}, soil density = 1.38 kg dm^{-3}, particle density = 2.63 kg dm^{-3}, total porosity = 0.48, calcium = 2.38 $cmol_c$ dm^{-3}, magnesium = 1.66 $cmol_c$ dm^{-3}, sodium = 0.23 $cmol_c$ dm^{-3}, potassium = 14.14 $cmol_c$ dm^{-3}, hydrogen = 5.69 $cmol_c$ dm^{-3}, aluminum = 5.69 $cmol_c$ dm^{-3}, organic matter = 20.38 g kg^{-1}, and pH = 4.8. Before sowing, the soil was limed to increase the base saturation to 70%, and fertilized according to the recommendations of Santos et al. (2022).



P - Precipitation; Tm - Mean air temperature; Tn - Minimum air temperature; Tx - Maximum air temperature; RH - Mean relative air humidity; E - Daily evaporation; DHS - Daily heat stroke obtained by radiometry; S - Sowing; Spr - Spray (Spr); PMC - Plant material collection for biochemical analysis

Figure 1. Weather conditions during the cultivation of cowpea plants

After raising the soil moisture to a maximum capacity for water retention, six seeds were sown per pot at a depth of 2 cm. Standard agricultural tillage practices were carried out to keep the crops free from weeds, disease, and pests. Plants were thinned 7 days after seedling emergence (DAE), retaining two plants per pot. Irrigation was performed at 25 DAE accounting for daily evapotranspiration (ETPi) measured using an evaporimeter (Soil Control Equipment). ETPi was quantified using Eq. 1 (Galvani et al., 1998):

$$ETPi = \left(\frac{0.28 \times Pi}{1 - W} \right) \quad (1)$$

where:

Pi - evapotranspiration in mm per day;

W - temperature estimate (W = 0.483 + 0.01 × T, T < 32 °C); and,

T - wet-bulb temperature.

Treatments were started at the V5 stage (25 DAE) and involved wetting the abaxial and adaxial sides of the leaves. Plant material used in the analysis was collected at the beginning of the R2 stage (61 DAE). The relative water content (RWC) of the leaves was calculated using Eq. 2, according to Merwad et al. (2018):

$$RWC = \left(\frac{FM - DM}{TM - DM} \right) \times 100 \quad (2)$$

where:

RWC - relative water content;

FM - fresh mass;

DM - dry mass; and,

TM - turgid mass.

The percentage of moisture in the leaves was calculated using Eq. 3, based on Slavick (1974):

$$\%U = \left(\frac{FM - DM}{FM} \right) \times 100 \quad (3)$$

where:

%U - percentage of moisture;

FM - fresh mass; and,

DM - dry mass.

For total soluble sugars determination (TSS), 200 mg of fresh leaves were macerated in 2.0 mL of 80% ethanol, transferred to microtubes, and placed in a 60 °C water bath for 20 min followed by 5 min centrifugation at 2,000 × g. The supernatant was collected and transferred to a test tube. This procedure was repeated twice to obtain 6 mL of extract. TSS was quantified using the phenol-sulfuric acid method described by Dubois et al. (1956). The samples were read using a spectrophotometer at a wavelength of 490 nm, and the results were expressed in μg of TSS g^{-1} of FM.

The total free amino acid extract (TFAA) was extracted from 250 mg of fresh tissue in 5 mL of sulfosalicylic acid. TFAA concentrations were determined using a modified version of the method described by Peoples et al. (1989), with 100 μL of extract, 400 μL of H_2O, 250 μL of citrate buffer solution (200 mM, pH = 5.0), and 250 μL of ninhydrin reagent (0.1 mmol L^{-1} KCN - potassium cyanide and 5% ninhydrin in methoxy ethanol). The tubes were then sealed, vortexed, and placed in a water bath at 100 °C for 15 min. The reaction was stopped by immersion in an ice bath. Finally, 1.5 mL of 50% ethanol (v/v) was added to the solution. After vortexing, the tubes were incubated for 20 min at room temperature. Absorbance was then read at 570 nm, and the results were expressed in μmol of TFAA mg^{-1} of FM. The same plant extract was used to quantify free proline levels according to Bates et al. (1973), with absorbance measured at 520 nm.

To quantify total water-soluble protein (TSP), a plant extract was prepared with 200 mg of fresh leaf tissue macerated in 2 mL of potassium phosphate buffer (50 mM, pH = 7.8), ascorbic acid (0.1 mM), EDTA (0.1 mM), and polyvinylpyrrolidone (0.3%). The extracts were then centrifuged at 2,000 × g for 20 min at 4 °C. The supernatant was transferred to microtubes and refrigerated at -20 °C. TSP was quantified using 23.4 μL of the plant extract and 700 μL of Bradford reagent (Bradford, 1976) based on absorbance at 595 nm and expressed in mg of protein g^{-1} of fresh mass.

The photoreduction inhibitory capacity of blue nitrotetrazolium chloride (BNC) by the enzymes present in the plant extracts was used to determine the activity of superoxide dismutase enzyme (SOD) (Giannopolitis & Ries, 1977). For this, aliquot samples (100 μL) were added to test tubes protected from light and containing the reaction medium (1,900 μL) composed of potassium phosphate buffer (100 mM, pH = 7.8), EDTA (0.1 mM), methionine (13 mM), and BNC (750 μM). The reaction was initiated by the addition of riboflavin (7 μM), and the tubes were transferred to a sealed reaction box with internal lighting (35 W) at room temperature. The samples were then maintained in this environment for 15 min. Absorbance was measured using a spectrophotometer at 560 nm.

Tubes kept in the dark were considered as controls (representing 0% of the inhibition of NBT-white from the dark), and the tubes kept under light and without the extract represented 100% of the inhibition of the NBT-white from the light. One unit of SOD was considered the amount of enzyme needed to inhibit 50% of the photoreduction of NBT compared to the clear white, and enzyme activity was expressed as AU min^{-1} mg^{-1} of protein.

Catalase enzyme activity (CAT) was measured by adding the enzyme extract (150 μL) to a quartz cuvette containing 1,950 μL of determination buffer (100 mM, pH = 7.0), 150 μL of extraction buffer (50 mM, pH = 7.5), and 750 μL of H_2O_2 solution (50 mM) (Tehrani & Moosavi-Movahedi, 2018). After stirring, the solution was analyzed using a spectrophotometer at 240 nm, and the decrease in absorbance was observed over a period of 2 min with readings taken every 10 s. The Beer-Lambert law was used in the calculations, as shown in Eq. 4:

$$A = \varepsilon \times b \times c \quad (4)$$

where:

A - decrease in absorbance (mean values in triplicate);

ε - molar extinction coefficient of hydrogen peroxide (39.4 mol cm^{-1});

b - light path; and,

c - enzyme concentration (mol L^{-1}). Catalase activity was expressed in μmol H_2O_2 min^{-1} mg^{-1} of protein.

By measuring the decrease in absorbance at 290 nm in a quartz cuvette, ascorbate peroxidase activity (APX) could be calculated based on ascorbate consumption (Nakano & Asada, 1981). The reaction medium (2.7 mL) was composed of potassium phosphate buffer (50 mM, pH = 6.0) and ascorbic acid (0.8 mM), to which 100 mL of enzymatic extract was added. The Beer-Lambert law was then applied (Eq. 4) with ascorbate (2.8 mM cm^{-1}) as the molar extinction coefficient. The final APX activity was expressed as nmol ascorbate min^{-1} mg^{-1} protein.

Analysis of variance was performed with a significance threshold of $p \leq 0.05$, and Tukey tests ($p \leq 0.05$) was performed to compare the means of PRO and SA. The F test ($p \leq 0.05$) was used to evaluate the effects of water replacement. Statistical tests were performed using SISVAR 5.6 software.

Results and Discussion

Water restriction and the use of PRO and SA, hereafter referred to as attenuators, alone or in combination, had significant effects on TSS, TFAA, PRO, and TSP, but did not affect RWC and %U (Table 1). Similarly, Coelho et al. (2014) reported no significant reduction in leaf moisture content when the cowpea variety 'Pele de Moça' was subjected to water and saline stress, indicating tolerance to dehydration.

When the plants were exposed to unfavorable water conditions, osmotic adjustment might have been a factor contributing to the preservation of adequate RWC and %U. The accumulation of compatible osmolytes allows water absorption by plant tissues, ensuring that their physiology remains stable under stress (Melo et al., 2022a).

The observed responses depended on the water replacement regimes (Table 1). For example, the TSS concentration increased by 58.54% in plants under water stress (Figure 2A) but only in the absence of the attenuators. The accumulation of carbohydrates may be associated with osmotic adjustment, a mechanism used by plants when water is scarce. The cytoplasmic increase in TSS may also be linked to starch breakdown, which can increase the osmotic adjustment of plants (Coelho et al., 2018).

Although not statistically significant, when PRO was employed separately or in combination with SA, the TSS concentrations under the W100 irrigation treatment were lowered by 17 and 26%, respectively, compared to the W50 irrigation in the control treatment. Under the W50 regime, leaf TSS content was reduced by 34% with SA treatment, 60% with PRO treatment, and 57% with PRO + SA treatment (Figure 2A).

The application of SA enhances nitrogen fixation by stimulating the activity of enzymes that participate in nitrogen metabolism, such as nitrate reductase, especially in legumes (Melo et al., 2022b). Additionally, it has been suggested that the application of SA may be an important aspect of legume-rhizobia symbiosis (Andrade et al., 2021). Thus, nitrogen absorption is particularly important, as it constitutes amino acids, proteins, enzymes, vitamins, alkaloids, and some growth hormones. In the present study, the application of SA increased the PRO and TSP concentrations under the W100 treatment.

Aires et al. (2022) reported a reduction in stomatal conductance, intracellular CO_2 content, and chlorophyll fluorescence in plants subjected to 1.3 mM SA. Thus, using a dose four times greater than the dose of SA studied by those researchers, it was observed that the reduction in TSS levels may be related to the inhibitory effects of SA on photosynthetic parameters (Melo et al., 2022a).

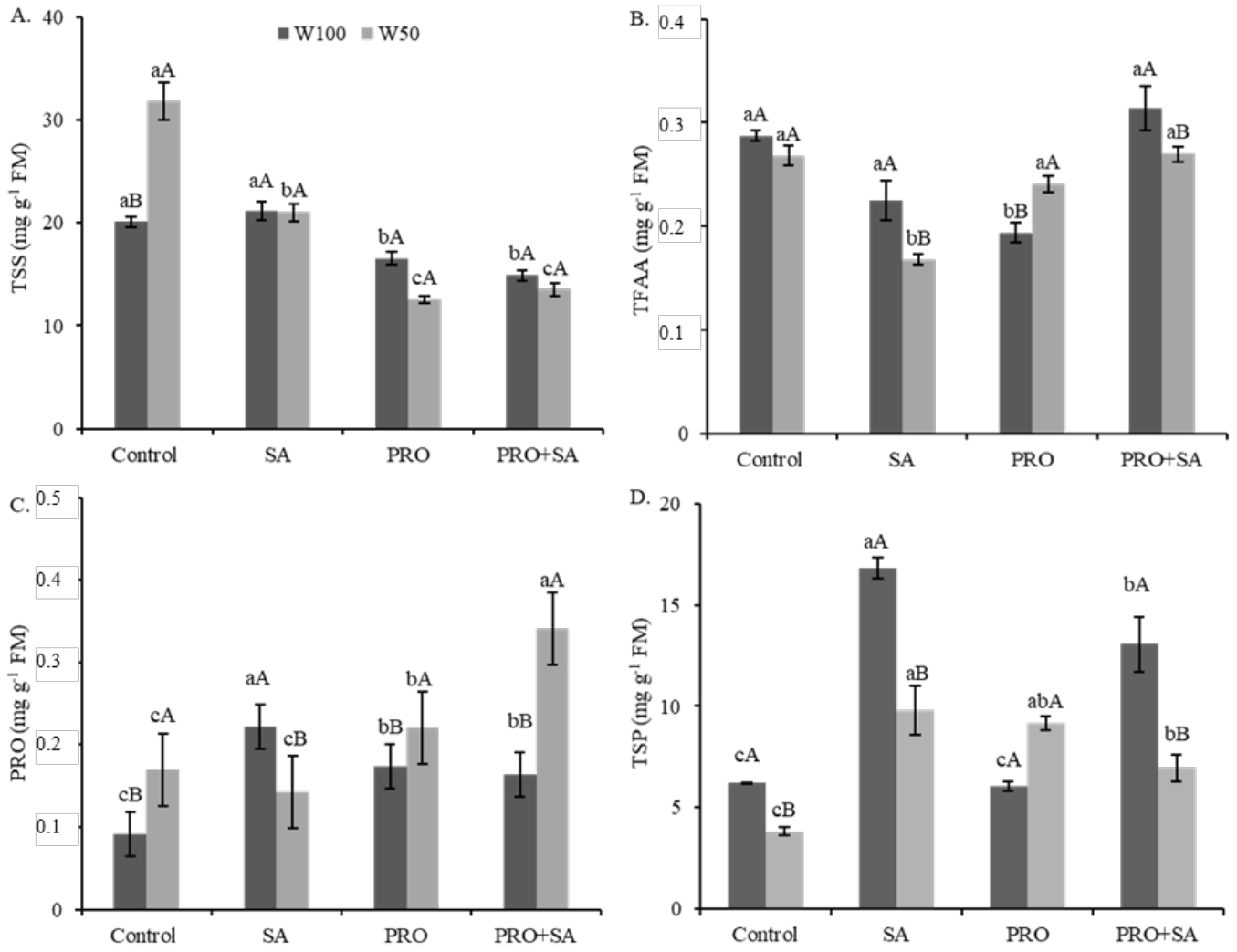
PRO, both separately or in combination with SA, promoted TSS reduction, particularly when plants were subjected to water deficit. When applied to leaves, this amino acid reduces stomatal opening (Bekka et al., 2018), which limits the synthesis of compounds derived from photosynthesis owing to lower CO_2 uptake, and induces plant osmoprotection. Under the conditions of the current study, stomatal closure may have reduced the excessive loss of water through transpiration. This is supported by the fact that the relative water content of the leaves remained unchanged despite water deficit.

There were no significant differences in TFAA levels between the W100 and W50 treatments and the control (Figure 2B). It is possible that TFAA does not participate in osmotic adjustment of the 'Paulistão variety' of cowpea under the experimental conditions evaluated here. However, under the W50 irrigation regime, the application of SA separately and in combination with PRO reduced TFAA levels by 25 and 14%, respectively, compared to the W100 regime. In contrast, when compared to the W100 regime irrigation, the isolated application of PRO increased TFAA levels by 24% under the W50 irrigation regime (Figure 2B). Therefore, the observed

Table 1. Summary of the analysis of variance for soluble sugars (TSS), total free amino acids (TFAA), proline (PRO), total soluble proteins (TSP), relative water content (RWC), and percentage of moisture in the leaves (%U) of cowpea plants under two conditions of water replacement and doses of salicylic acid and proline (attenuators), during the V4 phenological stage

SV	DF	Mean squares					
		TSS	TFAA	PRO	TSP	RWC	%U
Attenuators (A)	3	318.799**	0.999**	1.912**	124.017**	123.082^{ns}	47.77^{ns}
Water replacement (W)	1	24.242*	0.157*	2.309**	96.498**	274.276^{ns}	94.7^{ns}
A × W	3	121.843**	0.251**	2.104**	53.042**	68.90^{ns}	26.26^{ns}
Residual	32	3.836	0.035	0.048	2.672	84.518	29.37
CV (%)		10.35	11.06	13.22	18.19	12.26	6.51

SV - Sources of variation; DF - Degrees of freedom; CV (%) - Coefficient of variation; ** and * - Significant ($p \leq 0.01$), ($p \leq 0.05$), respectively, and ^{ns} - Not significant ($p > 0.05$) by the F test



The same lowercase letters for the same water replacement regime indicate no difference among the attenuators according to Tukey tests ($p \leq 0.05$). The same uppercase letters indicate no significant differences between water replacement regimes within each attenuator based on the F-test ($p \leq 0.05$). Vertical bars represent the standard error of the mean (n = 5)

Figure 2. Concentrations of total soluble sugar, TSS (A); total free amino acid, TFAA (B); proline, PRO (C); and soluble protein, TSP (D) in cowpea leaves as a function of water replacement (W100 and W50) and foliar application of salicylic acid (SA) and proline (PRO)

decrease in TFAA levels under the PRO + SA treatment can be attributed to SA activity.

When the W100 and W50 irrigation regimes were compared to the control and water restriction conditions, the PRO concentrations of the cowpea leaves were enhanced by 85% (Figure 2C). This increase may have been caused by the action of proteinases, which maintain leaf water potential when the concentration of PRO in the cytoplasm rises. This allows plants to control osmotic pressure and prevent dehydration (Carvalho et al., 2019; Jayawardhane et al., 2022). PRO accumulation under water stress mainly results from the activation of biosynthetic enzymes in many plant species (Szabados & Savouré, 2010). In cowpeas, this varies greatly according to genotype, leaf age, and phenological stage (Zegaoui et al., 2017; Santos et al., 2022).

When PRO and PRO + SA were applied, amino acid concentrations under the W50 irrigation treatment increased by 27 and 107%, respectively, compared to the W100 irrigation regime. Furthermore, under the W50 irrigation regime, PRO and PRO + SA application increased amino acid concentrations by approximately 30 and 101%, respectively, relative to the control treatment without attenuator application (Figure 2C).

In the current study, the observed mean values were consistent with those reported by Merwad et al. (2018), who also investigated the effects of PRO on plants; compared to an irrigated control treatment, these researchers found an 18% increase in the accumulation of endogenous PRO in plants subjected to water deficit. Accordingly, exogenous PRO plays a vital role in plant adaptation under adverse conditions, as this osmoprotectant induces improvements in plant growth and production.

Under the W50 and control treatments, there was a 38% decrease in the TSP concentration compared to the W100 irrigation treatment (Figure 2D). This is now unexpected given that a shortage of water causes several changes in cellular metabolism including proteolysis, reduced protein synthesis, disturbance to amino acid metabolism, and an increase in protease activity (Carvalho et al., 2019). More specifically, compared to the control, under the W50 treatment, TSP concentrations increased by 173, 140, and 82% in the cowpea plants treated with SA, PRO, and PRO + SA, respectively, under

water-stress conditions. The increase in TSP concentrations in the plants subjected to water stress and PRO application indicates that this amino acid is involved in protein integrity, by limiting denaturation under stress conditions (Zegaoui et al., 2017).

An increase in protein concentrations were also observed in the SA treatment under the W50 irrigation regime relative to the control treatment and with the same water replenishment rate. This is most likely due to the fact that SA is a cellular signal that triggers numerous responses that mitigate the negative effects of environmental stresses (Araújo et al., 2018). Lee et al. (2019) and Melo et al. (2022a) also suggested that cellular protection against free radicals may lead to a reduced response to oxidative stress, which might explain the decrease in stronger enzyme activity observed in attenuator-treated plants under the W50 irrigation regime.

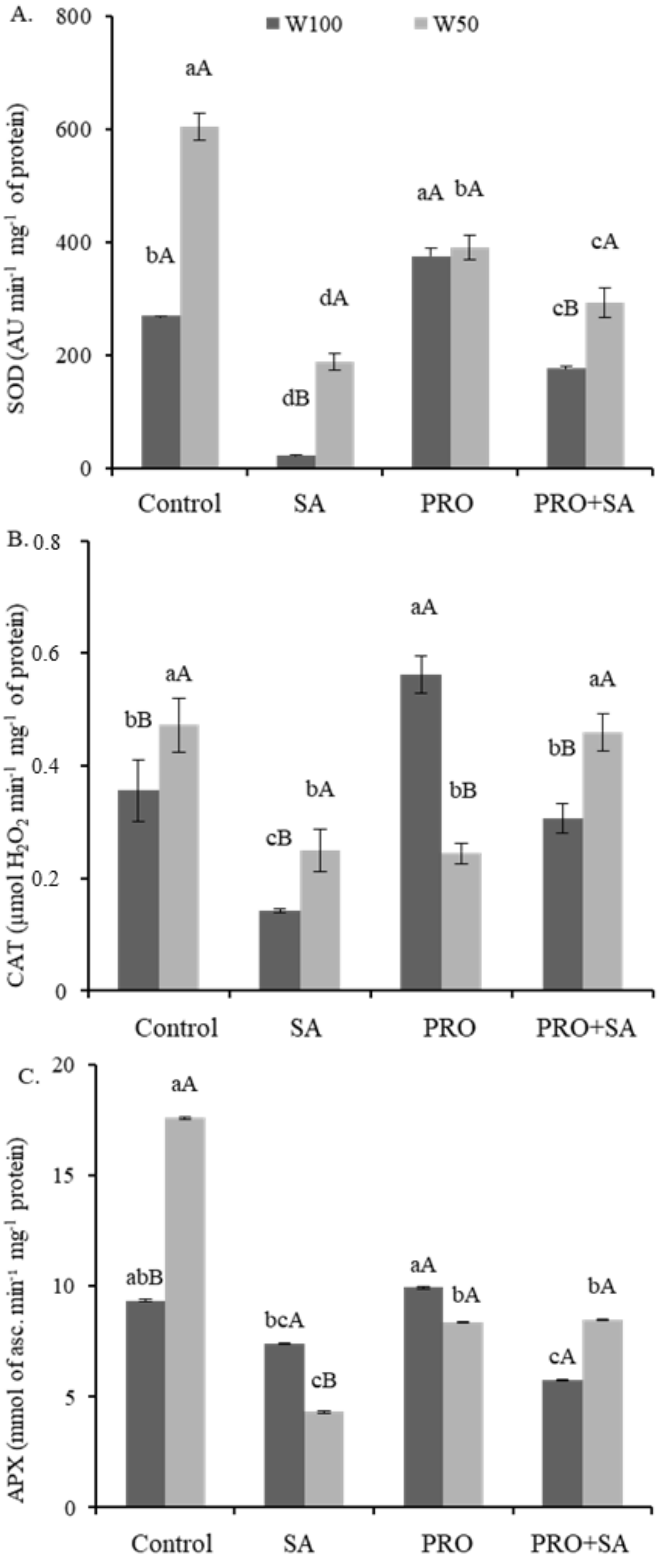
In the absence of attenuators, SOD, CAT, and APX enzyme activity increased by 124, 32, and 88%, respectively, under the W50 water replacement regime compared to the W100 regime (Figures 3A, B, and C). An increase in the activity of antioxidative complex enzymes under water-stress conditions is considered a common mechanism of oxidative stress response that helps eliminate reactive oxygen species. This process contributes to the maintenance of the integrity and stability of membranes and cellular structures, enabling osmoregulation (Melo et al., 2022a).

Except for the PRO + SA treatment and CAT, attenuator application under the W50 irrigation regime led to lower enzyme activity relative to the control treatment (Figures 3A, B, and C). For example, SOD activity was reduced by 68, 35%, and 51% after the addition of SA, PRO, and PRO + SA, respectively (Figure 3A).

Under the W50 regime, CAT activity in treated plants was reduced by 47 and 48% for SA and PRO treatments, respectively, relative to the control treatment (Figure 3B). The equivalent reductions for APX enzyme activity were 75, 52, and 52%, respectively (Figure 3C). These decreases in enzymatic activity may have been prompted by the removal of ROS in the cowpea plants under the W50 water replacement regime, either directly, through compounds acting in a non-enzymatic way, or via earlier enzyme activity, leading to better preservation of the homeostatic levels of these molecules. According to Lee et al. (2014), Sachdev et al. (2021), and Mekonnen et al. (2022), these mechanisms reduce the adverse effects of environmental stresses and ensure the integrity of cellular structures.

When comparing the W100 and W50 regimes for the SA-treated plants, a 700% increase in SOD activity and an almost 75% increase in CAT enzyme activity was observed (Figures 3A and B). In contrast, with SA treatment, APX activity decreased by 21% (Figure 3C). The increase in SOD and CAT enzyme activity following SA treatment supports the results of Dutra et al. (2017), who found that SA, which acts as an abiotic stress attenuator, is a significant promoter these enzymes in cowpeas under water stress. In addition, the activity of APX, which is involved in the removal of H_2O_2 from cells, decreased when SA was applied. This might be related to suppression resulting from the significant increase in CAT activity, which is also involved in the elimination of H_2O_2 (Melo et al., 2022a).

When foliar PRO spray was applied to the plants subjected to the W50 water replacement regime, SOD and APX activity



The same lowercase letters for the same water replacement regime indicate no difference among the attenuators according to Tukey tests ($p \leq 0.05$). The same uppercase letters indicate no significant differences between water replacement regimes with each attenuator based on the F-test ($p \leq 0.05$). Vertical bars represent the standard error of the mean ($n = 5$)

Figure 3. Superoxide dismutase, SOD (A); catalase, CAT (B); and ascorbate peroxidase, APX (C) activity in cowpea leaves as a function of water replacement (W100 and W50) and foliar application of salicylic acid (SA) and proline (PRO)

did not vary significantly compared to the W100 treatment (Figures 3A and C). However, PRO application reduced CAT activity by 56% in the plants subjected to the W50 water replacement regime relative to the W100 (Figure 3B). In

addition to enzymes, other compounds, such as compatible osmolytes that act as osmotic adjusters, may also be involved in the non-enzymatic removal of ROS and free radicals (Sachdev et al., 2021; Santos et al., 2022).

Conclusions

1. The application of SA increased osmoregulator synthesis, protein concentrations, and modulated antioxidant enzyme activity in cowpea plants subject to water stress.

2. PRO concentrations increased synergistically in plants treated with PRO and SA, particularly in plants subject to a 50% of water replacement regime.

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