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RESEARCH PAPER

Salicylic acid and antitranspirant polymer mitigate the effects of water stress on the growth and yield of cowpea

Anderson Carlos de Melo Gonçalves[1], Caio da Silva Sousa[2], Alex Serafim de Lima[2], Toshik Iarley da Silva[3], José de Anchieta Alves de Albuquerque[1], Evandro Franklin de Mesquita[2], Thiago Jardelino Dias[4], Walter Esfrain Pereira[4], José Maria Arcanjo Alves[1], Leandro Torres de Souza[1], Aloisio Alcantara Vilarinho[5]

1 *Universidade Federal de Roraima, Campus Cauamé, Boa Vista, Roraima, Brazil*
2 *Universidade Estadual da Paraíba, Campus IV, Catolé do Rocha, Paraíba, Brazil*
3 *Universidade Federal de Campina Grande, Centro de Ciências e Tecnologia Agroalimentar, Pombal, Paraíba, Brazil*
4 *Universidade Federal da Paraíba, Campus II, Areia, Paraíba, Brazil*
5 *Empresa Brasileira de Pesquisa Agropecuária, Centro Nacional de Pesquisa de Trigo, Passo Fundo, Rio Grande do Sul, Brazil*

Corresponding author: Anderson Carlos de Melo Gonçalves (anderson.agroufpb@yahoo.com)

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Abstract

Water stress can limit the growth and production of cowpea (*Vigna unguiculata* (L.) Walp.), necessitating the use of strategies that induce plant tolerance to mitigate its effects on the crop. This includes the application of salicylic acid (SA) and antitranspirant polymer (AP). The objective of this study was to evaluate the effect of SA and AP applications on the growth and production of cowpeas subjected to water stress. The experiment was conducted in field conditions at Sítio Boqueirão, Catolé do Rocha, Paraíba, Brazil. The experimental design used was randomized blocks, comprising 10 treatments with four replications. The evaluated treatments included nine combinations generated according to the Central Composite Design experimental matrix, involving five doses of SA and five doses of AP applied to plants under water stress, along with an additional treatment (without water stress and application of SA and AP). The assessed variables included morphological parameters and yield. The data were subjected to canonical correspondence analysis and confidence ellipses. The application of SA and AP increased growth (summer and spring) and productivity (summer). However, in spring, there was a decrease in productivity and an increase in the number of days until the initiation of pod harvesting in cowpeas under water stress.

Keywords

Beta hydroxy acid, Phytohormones, Plant hormones, Monomers, *Vigna unguiculata*, Water scarcity

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important leguminous food crop that grows in tropical and subtropical regions worldwide. It is cultivated globally, with a planted area of 14.5 million hectares and an estimated production of 6.2 million tons (Kebede and Bekeko 2020).

Cowpea cultivation was historically limited, mainly in the Northeast and North of Brazil, with low technology adoption. In recent years, its cultivation has been expanding rapidly to other regions of the country, particularly in the Central-West region. Despite this trend, there is a lack of knowledge dissemination and technology transfer regarding cowpea cultivation (Vale et al. 2017).

Stress on plants has increased dramatically since climate change began, restricting their ability to grow and produce (Khan et al. 2020). Nevertheless, many plants exhibit plasticity in their physiology and development as a means of ensuring their survival in adverse conditions (Mickelbart et al. 2015). Unfavorable environmental conditions for plants encompass both biotic factors (insects, parasites, bacteria, fungi, and viruses) and abiotic factors (salinity, flooding, drought, freezing, heavy metals, cold, and heat), with the abiotic factors being the most concerning as they threaten global food security by restricting the growth and production of crops (Zhu 2016). Among these, water stress has one of the most significant impacts on agricultural crops, representing the most crucial abiotic stress worldwide, severely constraining plant growth and development and thereby reducing crop productivity (Fita et al. 2015).

Understanding plant mechanisms in response to water stress is crucial for predicting plant functionality during water scarcity (Sun et al. 2020). Mitigation strategies are necessary to enhance plant resistance to water stress and alleviate its effects on growth and crop production (Vurukonda et al. 2016). One alternative that can be considered is the use of plant hormones, as they regulate plant responses to biotic and abiotic stresses (Peleg and Blumwald 2011). Among them, salicylic acid (SA) is a highly promising compound, playing a significant role in plant adaptation to growth-limiting conditions. Its exogenous application may represent a novel approach to inducin tolerance to both biotic and abiotic stresses (Kang et al. 2014).

Another strategic alternative to mitigate abiotic stresses is the use of polymers, which play a role from our food to clothing. These polymers can be organic, or inorganic, natural, or synthetic (Ojeda 2013). Among the diverse range of polymers, especially those used in agriculture, anti-transpirant polymers deserve attention as a beneficial alternative to reduce water losses through transpiration and maintain water potential in plants cultivated under stress conditions (Dass and Bhattacharyya 2017). The aim of this study was to evaluate the effect of applying salicylic acid and anti-transpirant polymer on the vegetative growth, and production of cowpea crops subjected to water stress.

Material and methods

Experiment location

The experiment was conducted under field conditions at Sítio Boqueirão, in the rural area of Catolé do Rocha, Paraíba, Brazil, during two cropping cycles. The first cycle took place between December 2019 and February 2020 (summer), and the second between September and November 2020 (spring). The experimental area is located at 6°21'50"S, 37°40'59"W, with an altitude of 220 m.

The municipality's climate, classified according to Thornthwaite, is of type Ds2A'b'1, characterizing it as a semi-arid climate, termed megathermic, with maximum temperatures reaching 35 °C and minimum temperatures of 19 °C. The average annual precipitation is 800 mm, distributed across two seasons: a rainy season with irregular precipitation mainly between February and April, and a dry season with higher water deficiency in the summer, resulting in an average annual evapotranspiration (ET) of 1,598 mm. Of this ET, 68.2% occurs during the summer (Dantas et al. 2018; Melo et al. 2018).

Climate data (Fig. 1) were recorded using a digital thermo-hygrometer (HT-600 Instruthermr®), placed in a shelter at the center of the experimental area, 1.5 m above

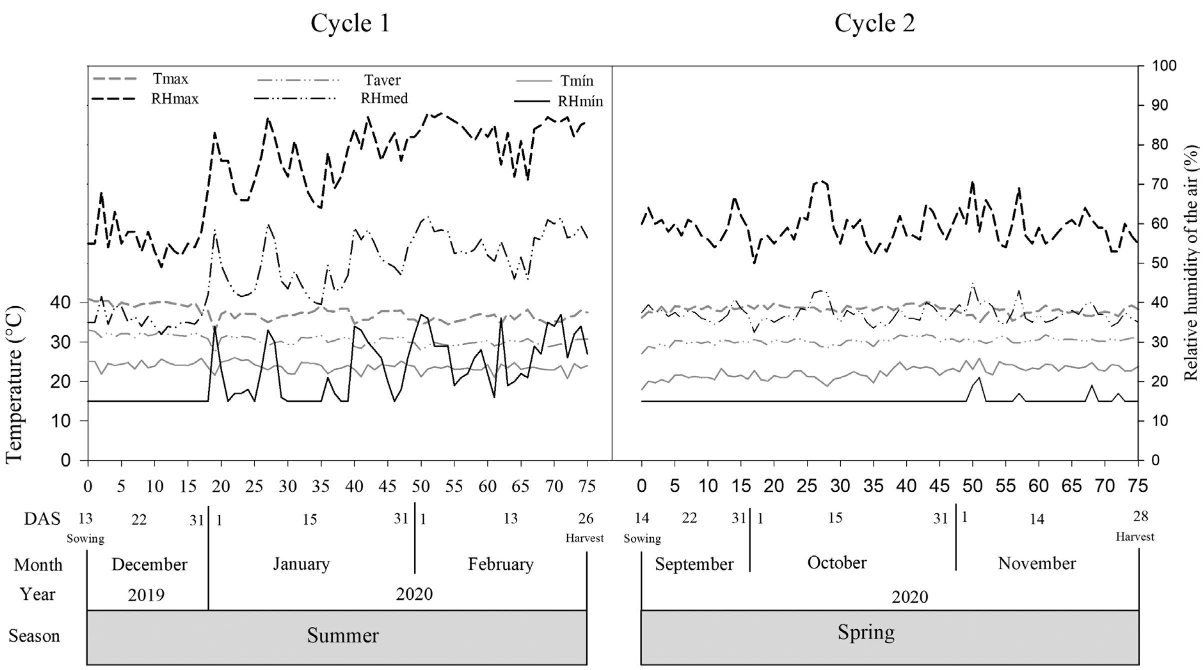

Figure 1. Maximum (Tmax), average (Taver), and minimum (Tmin) air temperatures in °C; maximum (URmax), average (URmed), and minimum (URmin) relative humidity in %, during the experimental period in summer (Cycle 1) and spring (Cycle 1) of 2020 in the Catolé do Rocha region, Paraíba, Brazil.

Table 1. Chemical and physical characteristics of the soil used in the experiment in the 0 to 20 cm depth layer.

Chemical characteristics												
pH	P	K^+	Na^+	H+Al	Al^{+3}	Ca^{+2}	Mg	SEB	CEC	V	m	OM
	— (mg dm^{-3}) —		— (cmol dm^{-3}) —							— (%) —		(g kg^{-1})
7.25	5.35	183.77	2.56	0.00	0.00	4.88	2.18	10.09	10.09	100	0,00	1,67
Physical characteristics												
Sd	Pd	TP	FC	PWP	Sand	Silt	Clay		Textural classification			
— (g cm^{-3}) —		($m^3 m^{-3}$)	— (g kg^{-1}) —						- Embrapa -		- Atterberg -	
1.61	2.72	0.40	131.1	48.2	700.8	221.2	78		Average		Sandy loam	

pH = water: 1:2.5; P, K, Na = Mehlich Extractor; H+Al = 0.5 M Calcium Acetate Extractor, pH 7.0; Al, Ca, Mg = 1 M kCl Extractor; SEB = Sum of Exchangeable Bases; CEC = Cation Exchange Capacity; V = Base Saturation; m = Aluminum Saturation; OM = Organic Matter; Sd = Soil Density; Pd = Particle Density; TP = Total Porosity; FC = Field Capacity; PWP = Permanent Wilting Point.

ground level. Data were collected daily in the late afternoon throughout the experimental period.

The soil was classified as Eutrophic Fluvic Neosol with a sandy loam texture (Santos et al. 2018). The chemical and physical characteristics of the soil in the 0–20 cm depth layer (Table 1) were analyzed following the methods outlined by Silva (2009) for chemical analysis and Santos et al. (2018) for physical analysis.

The cowpea seeds used were from the landrace cultivar 'Pingo-de-Ouro' cultivated in a no-tillage system. After desiccation of the previous crop (corn) and weeds using glyphosate (N-[phosphonomethyl]-glycine) at a dose of 4.075 kg ha^{-1}, recommended for areas with the occurrence of *Portulaca oleracea*. The seeds were mechanically sown, with 10 seeds per linear meter in double-row spacing of 0.60 × 0.30 × 0.20 m. After emergence stabilization, thinning was performed, leaving five plants per linear meter, resulting in a population density of 111,111 plants per hectare, according to Moreira et al. (2016).

Both the planting and top-dressing fertilization, for both summer and spring planting, were based on soil chemical analysis and the fertilization recommendation manual for the state of Pernambuco (IPA 2008). The planting fertilization was broadcast using doses of 20, 20, and 20 kg ha^{-1} of N, P_2O_5, and K_2O, respectively. Top-dressing fertilization was applied using a broadcast method with a dose of 20 kg ha^{-1} of N at 20 days after planting. The sources of N, P_2O_5, and K_2O were urea (for both planting and top-dressing), single superphosphate, and potassium chloride, respectively.

Preventive control of aphids (*Aphis craccivora*) and pod borers (*Michaelus jebus*) was carried out using the insecticides Imidacloprid and Methomyl at doses of 150 g/100 kg of seeds and 215 g ha^{-1}, respectively. Although there was an occurrence of *Fusarium oxysporum*, it did not reach the control threshold. Weed control was performed 15 days after emergence (DAE) using the herbicide ethofumesate at a dose of 82.5 g ha^{-1}.

For water management, the localized irrigation method was employed using a drip system installed between the rows with a narrower spacing (30 cm). The drip tape had a diameter of 16 mm, a wall thickness of 200 microns, emitter spacing of 20 cm, and a maximum emitter flow rate of 3.8 L h-1, although its operational flow rate was 3.32 L h^{-1}. These characteristics provided a wetted area percentage (P) of 50%. The chemical analysis data of the water used in the experiment for irrigation purposes is presented in Table 2.

Regarding water management (Fig. 2), the calculation of the gross irrigation depth (L_b) considered, first, the reference evapotranspiration (ETo, mm day^{-1}), the product of daily evaporation from the Class A pan, and its coefficient (Kp = 0.75). By multiplying the ETo value with the crop coefficient (Kc) recommended by Bastos et al. (2008) and subtracting values for any precipitation, the net irrigation depth (LL, mm day^{-1}) was calculated. This value was then divided by the irrigation system efficiency (Ef = 0.95), resulting in the gross irrigation depth (L_b), applied daily in the early hours of the day, except during the period and plots subjected to water interruption treatments.

From 25 DAE, irrigation of the plots subjected to water stress was interrupted until the soil matric potential at a depth of 0.40 m indicated an absence of available water (Ribeiro et al. 2019). The determination of soil matric potential (Ψ_m) during this stage was monitored by an analogic tensiometer randomly installed in the area at the beginning of the irrigation interruption.

The harvesting system was manual, carried out when the physiological maturity of the pods was observed. After harvesting, the pods were manually threshed, cleaned by ventilation, exposed to direct solar drying until reaching the standard commercial moisture content (13%), and stored in a refrigerator at a constant temperature of 10 °C (Bortolotto 2005).

Table 2. Chemical properties of the water used in the experiment for irrigation purposes.

Chemical properties of the water												
pH	EC	Ca^{++}	Mg^{++}	Na^+	K^+	SO_4^{-2}	CO_3^-	HCO_3^-	Cl^-	SAR	PSI	Classification
	(dS m^{-1})	— ($mmol_c$ L^{-1}) —										
6,22	0.32	0.80	0.65	4.13	0.11	0.29	0.00	2.90	2.20	4.85	***	C_1S_1

EC = Electrical Conductivity; SAR = Sodium Adsorption Ratio; PSI = Paper Spray Ionization.

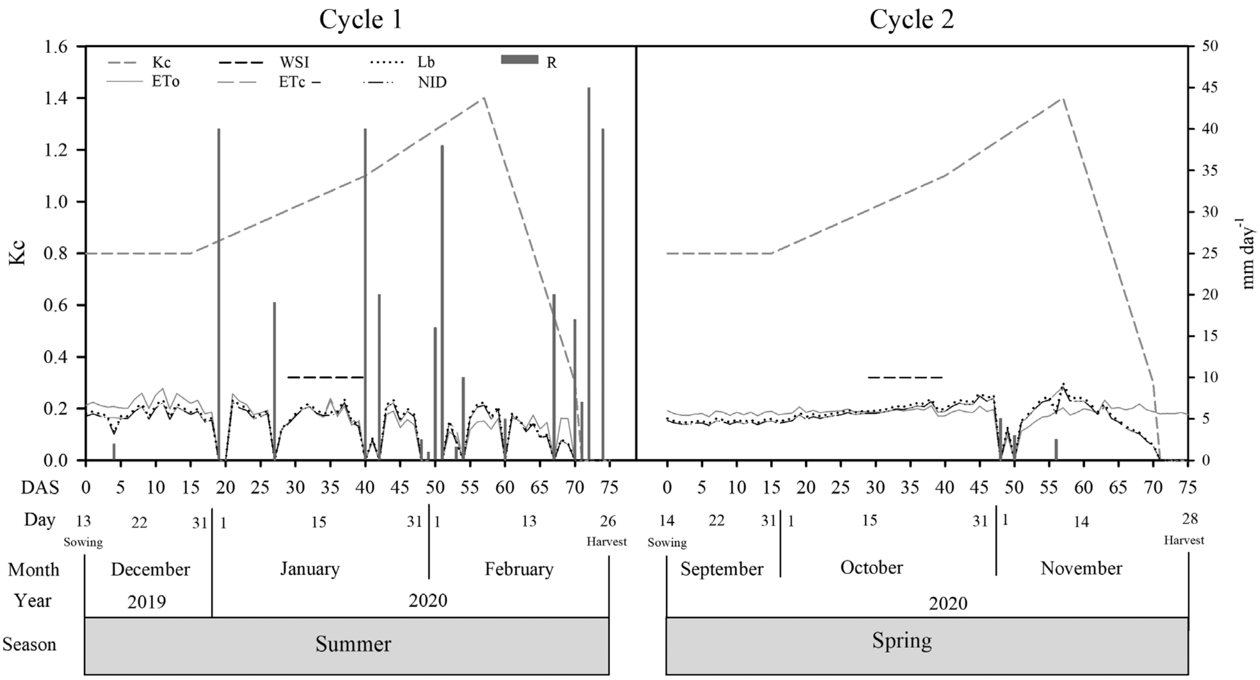

Figure 2. Water management for cowpea crops subjected and not subjected to water stress over the days after planting. Crop coefficient (Kc), reference evapotranspiration (ETo), crop evapotranspiration (ETc), rainfall (R), net irrigation depth (NID), gross irrigation depth (Lb), and water stress interval (WSI).

Experimental design

The experimental design employed was a randomized complete block with 10 treatments and four replications. The factors assessed included five doses of salicylic acid (SA) and five doses of antitranspirant polymer (AP) combined according to the Central Composite Design, resulting in nine combinations (0.29 and 0.29; 1.71 and 0.29; 0.29 and 1.71; 1.71 and 1.71; 1.00 and 0.00; 1.00 and 2.00; 2.00 and 1.00; 0.00 and 1.00; 1.00 mM and 1.00%) applied at the beginning of water interruption (25 DAE). An additional treatment (control) was included, which was free from water stress and the application of SA and AP doses. The antitranspirant polymer used was HumigelPlus®, composed of 2% nitrogen, 4% CaO, 2.2% MgO, and 1.4% fulvic acids (Tecniferti 2018).

The application of SA and AP doses was carried out separately in the early hours of the day in the corresponding experimental units. SA was diluted in 20 mL of ethyl alcohol and water (Table 2) with 0.05% neutral detergent as a surfactant, applied on the first day of water stress initiation (25 DAE). AP was diluted in water with 0.05% neutral detergent as a surfactant and applied on the second day of water stress (26 DAE). A manual sprayer was used for dose application, spraying the SA and AP solution until reaching the point of maximum leaf saturation (runoff).

The experimental plots consisted of 12 single rows (6 double rows) with a length of 4 m. The useful area (10.8 m^2) was composed of 8 central single rows (4 double rows), excluding 0.5 m from the front ends, while the remaining lines formed the border.

Variables evaluated

The plant height (PH) was measured using a graduated ruler in centimeters, taking the distance from the plant's base to its apex. Stem diameter (SD) was determined at the base of the plant using a digital caliper (precision 0.1 mm). The number of leaves (NL) was determined by counting all the leaves on the plants, excluding senescent leaves. The number of nodes on the stem (NNS) was determined by counting from the first true node. The number of branches per plant (NBP) was determined by counting the number of branches emitted by each plant. These variables were assessed in five weekly evaluations starting from the first day of water stress and treatment application (25 DAE) on three designated plants marked with stakes and different-colored ribbons.

The onset of maturation (OM) was determined by considering the number of days elapsed between seeding and the appearance of the first mature pods, marked by a change in pod color from green to reddish (a characteristic of the cultivar). The number of days until the start of harvest (NDH) was determined after observing pods in their final stage of physiological maturation in more than 50% of the plants.

The dry grain mass per plant (DGMP) was determined by multiplying the number of pods per plant by the average mass of grains from 5 dried pods. Grain yield (GY) was estimated in kg ha^{-1}, considering the mass of dried grains adjusted for 13% humidity. Green pod yield (GPY) was calculated by multiplying the mass of dried pods by the ratio of the average mass of green pods to dried pods from five pods each, with the result estimated in kg ha^{-1}. Green grain yield (GGY) was calculated by multiplying the mass of dried grains by the ratio of the average mass of green grains to dried grains from five pods each, with the result estimated in kg ha^{-1}.

Data analysis

The data were subjected to canonical correspondence analysis and confidence ellipses ($p \leq 0.01$) to study the interrelation between variables and factors using the candisc package (Friendly and Fox 2017). The R statistical program (R Core Team 2021) was employed for conducting the statistical analyses.

Results

According to the analysis of growth variables, at 25 DAE, there was a slight dispersion in relation to the variation of treatment values, both in the summer cultivation (Fig. 3A) and in the spring cultivation (Fig. 3B). This is because the evaluation was performed on the same day as the application of treatments, and there was not enough time for the expression of their effects. At 32 DAE, the analysis of growth variables, including plant height (PH), stem diameter (SD), number of leaves (NL), number of nodes on the stem (NNS), and number of branches per plant (NBP), showed the highest values in the treatment where plants were grown without water stress (S0P0) in both summer cultivation (Fig. 3C) and spring cultivation (Fig. 3D). This was followed by the treatment where plants were grown under water stress with the application of 1 mM of SA and 1% of AP (S1P1) in the variable PH and, in the other variables, 1.71 mM of SA and 0.29% of AP (S1.71P0.29), in the summer cultivation. In the spring cultivation, the highest values for all variables were observed in the treatment where plants were grown without water stress (S0P0).

At 39 DAE, plant height (PH), stem diameter (SD), number of nodes on the stem (NNS), number of leaf (NL), and number of branches per plant (NBP) showed the highest values in the treatment where plants were cultivated without water stress (S0P0) in both crops, followed by the treatment where plants were grown under water stress with the application of 0.29 mM of SA and 1.71% of AP (S0.29P1.71) in the summer crop (Fig. 3E) and 1 mM of SA and 1% of AP (S1P1) in the spring crop (Fig. 3F).

At 46 DAE, PH, SD, NNS, and NL presented the highest values in the treatment where plants were cultivated without water stress (S0P0) in both crops, followed by the treatment where plants were grown under water stress with the application of 0.29 mM of SA and 1.71% of PH (S0.29P1.71) in the summer crop (Fig. 3G) and 1.71 mM of SA and 1.71% of AP (S1.71P1.71) in the spring crop (Fig. 3H).

The highest values of NBP were not related to any of the treatments in the summer crop (Fig. 3G), but in the spring crop (Fig. 3H), it showed the highest values in the treatment where plants were cultivated without water stress (S0P0), followed by the treatment under water stress with the application of 1.71 mM of SA and 1.71% of AP (S1.71P1.71).

At 53 DAE, PH showed the highest values in the treatment where plants were cultivated without water stress (S0P0) in both crops, followed by the treatments where plants were grown under water stress with the application of 0.29 mM of SA and 1.71% of AP (S0.29P1.71) in the summer crop (Fig. 3I) and 2 mM of SA and 1% of AP (S2P1) in the spring crop (Fig. 3J).

The highest values of SD, NL, NNS, and NBP were not related to any of the treatments in the summer crop (Fig. 3I). However, in the spring crop (Fig. 3J), the highest values were related to the treatment where plants were cultivated without water stress (S0P0), followed by the treatment where plants were grown under water stress with the application of 2 mM of SA and 1% of PH (S2P1).

Regarding the analysis of production variables, it was observed that the highest values of dry grain yield (DGY), green pod yield (GPY), and green grain yield (GGY) were in the treatment where plants were cultivated without water stress (S0P0) in both cycles, followed by the treatment where plants were grown under water stress with the application of 2 mM of SA and 1% of AP (S2P1) in the summer crop (Fig. 4A). In the spring crop (Fig. 4B), the treatments under water stress showed the lowest values.

The dry grain weight per plant (DGP) showed the highest values in the treatment where plants were grown under water stress with the application of 1 mM of SA and 0% of AP (S1P0) in the summer crop (Fig. 4A). In the spring crop (Fig. 4B), the highest values were observed in the treatment where plants were cultivated without water stress (S0P0).

The beginning of maturation (BM) and the number of days to the beginning of harvest (NDBH) showed the highest values in the treatment where plants were grown under water stress with the application of 1.71 mM of SA and 1.71% of AP (S1.71P1.71) in the summer crop (Fig. 4A). In the spring crop (Fig. 4B), the highest values were observed in the treatment where plants were grown under water stress with the application of 0.29 mM of SA and 0.29% of AP (S0.29P0.29).

Discussion

The higher values of the variables in the treatment without water stress in cowpea plants are due to the greater water availability for the crop, allowing for maximum growth by meeting its water demand completely. This condition ensures a higher water influx and maintenance of cell turgor, providing suitable conditions for plant growth through cell division and expansion. These characteristics are diminished when plants are subjected to water stress (Locatelli et al. 2016; Taiz et al. 2017). The complete replacement of water transpired by the plant in the treatment without water stress was sufficient to meet the water demand of cowpea, resulting in higher yields in production components, particularly increasing productivity (Souza et al. 2020).

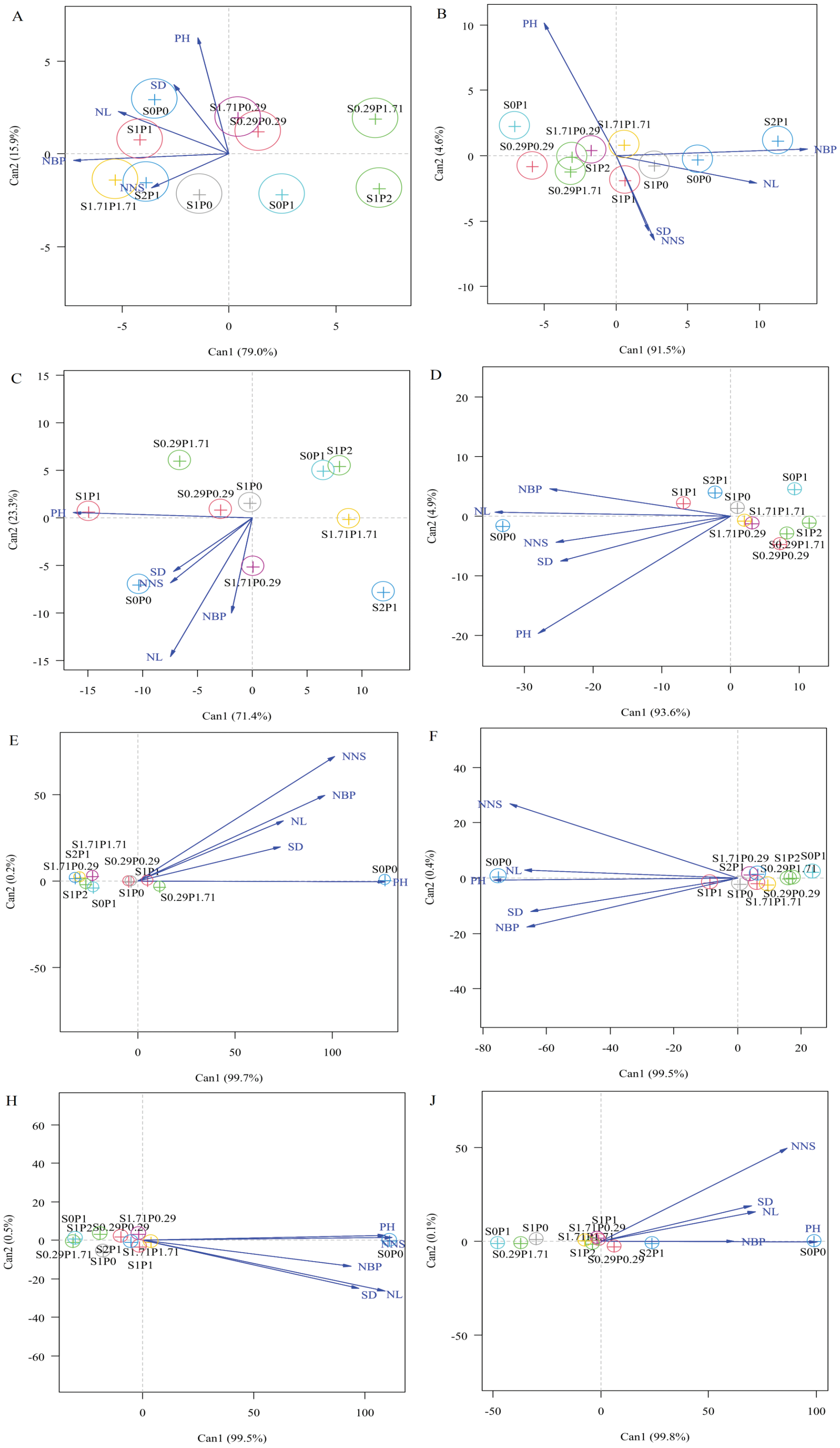

Figure 3. Canonical variable analysis with confidence ellipses were conducted among growth-related variables of cowpea (*Vigna unguiculata* (L.) Walp.) cultivated under non-water stress (S0P0) and water stress with the application of doses of salicylic acid and antitranspirant polymer, at respective doses of 0.29 and 0.29 (S0.29P0.29); 1.71 and 0.29 (S1.71P0.29); 0.29 and 1.71 (S0.29P1.71); 1.71 and 1.71 (S1.71P1.71); 1.00 and 0.00 (S1P0); 1.00 and 2.00 (S1P2); 2.00 and 1.00 (S2P1); 0.00 and 1.00 (S0P1); 1.00 mM and 1.00% (S1P1), in summer and spring crops, respectively, assessed at 25 (A and B), 32 (C and D), 39 (E and F), 46 (G and H), and 53 (I and J) days after emergence (DAE). Variables considered include plant height (PH), stem diameter (SD), number of leaf (NL), number of nodes on the stem (NNS), and number of branches per plant (NBP).

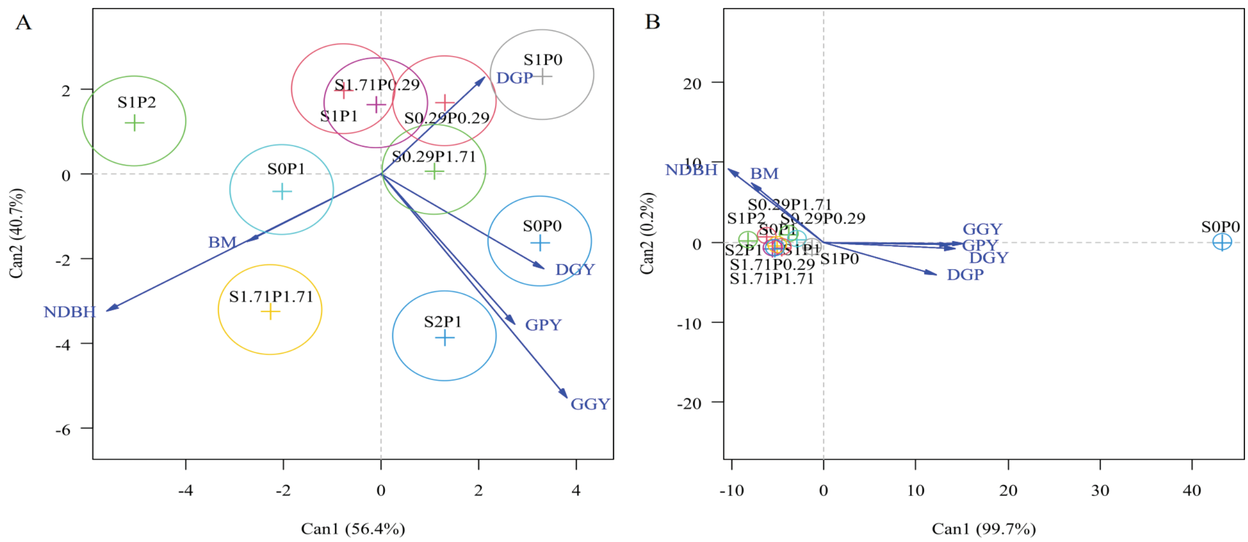

Figure 4. Canonical variable analysis and confidence ellipses among variables related to the production of cowpea (*Vigna unguiculata* (L.) Walp.) cultivated without (S0P0) and under water stress with the application of doses of salicylic acid and antitranspirant polymer, at respective doses of 0.29 and 0.29 (S0.29P0.29); 1.71 and 0.29 (S1.71P0.29); 0.29 and 1.71 (S0.29P1.71); 1.71 and 1.71 (S1.71P1.71); 1.00 and 0.00 (S1P0); 1.00 and 2.00 (S1P2); 2.00 and 1.00 (S2P1); 0.00 and 1.00 (S0P1); 1.00 mM and 1.00% (S1P1), in summer (A) and spring (B) crops. Beginning of maturation (BM), number of days to the beginning of harvest (NDBH), dry grain weight per plant (DGP), dry grain yield (DGY), green pod yield (GPY), and green grain yield (GGY).

The seed mass of cowpea decreases with the severity of water stress due to the partitioning of carbohydrates and assimilates in the developing seed. Under favorable conditions, seeds develop properly with high assimilation and carbohydrate accumulation, resulting in the maximum seed mass that the genotype can express (Yahaya et al. 2019).

Greater water availability has been shown to promote increased growth in cowpea genotypes under different irrigation strategies (Moreira et al. 2016). Conversely, when plants are subjected to water stress, limitations in their growth and production are observed, as seen in peppermint (*Mentha piperita*) subjected to normal irrigation and water stress in greenhouse and field experiments. Plants under water restriction conditions exhibited limited growth compared to those with an adequate water supply (Zade et al. 2019).

As observed, some treatments involving the application of salicylic acid (SA) have improved the performance of cowpea plants when exposed to water stress, possibly due to its role as an effective and environmentally friendly plant-protective and growth-regulating hormone. However, different concentrations can have stimulatory or inhibitory effects on plant development (Koo et al. 2020). SA plays a vital role in various plant signaling pathways, and several studies recommend its use in stress mitigation (Maruri-López et al. 2019).

Defensive effects of SA include the regulation of anti-stress processes and the recovery of growth processes after the end of stress, i.e., when the plant is rehydrated (Rady et al. 2017). As observed in this study, several other studies have demonstrated that growth and productivity were enhanced by SA application under water stress (El-Mageed et al. 2016; Semida et al. 2017; Hafeza and Sa-leiman 2017). Positive effects of SA have also been found in barley (Hordeum vulgare) cultivation with the application of 0.5 mM at 21 days after sowing (Abdelaal et al. 2020) and in the growth of rice seedlings (*Oryza sativa*) with the application of 1.00 mM to seeds by imbibition (Sohag et al. 2020).

Antitranspirants not only alleviate water loss but also enhance resistance to diseases, physiological processes, yield, and quality aspects in many plants (Ahmed et al. 2019). As observed in this study, the application of antitranspirant polymer contributed to the growth and production of cowpea under water stress. This effect can be associated with the reduction of transpiration rate and the alleviation of the detrimental effects of water stress on metabolic processes in leaf tissue (Hellal et al. 2020).

The use of antitranspirant polymer has positively influenced the growth and production of various crops, including rice, tomato (*Solanum lycopersicum*), and corn (*Zea mays*) under water stress. Therefore, it is recommended for these crops to mitigate the effects of water stress and also to conserve water application (Pirbalouti et al. 2017; Patel et al. 2019; El-Hadidi et al. 2020; Hassnain et al. 2020).

Conclusions

The vegetative growth and productivity of cowpea (*Vigna unguiculata* (L.) Walp.) are higher when the plants are cultivated without water stress, both in summer and spring. The application of salicylic acid and antitranspirant polymer at a dose of 0.29 mM and 1.71% in the summer and 2 mM and 1% in the spring improves the vegetative growth of cowpea plants cultivated under water stress. The application of salicylic acid and antitranspirant polymer at a dose of 2 mM and 1% in the summer increases the productivity of cowpea plants cultivated under water stress. The application of salicylic acid and antitranspirant polymer causes antagonistic effects on the productivity of cowpea crops subjected to water stress and delays the onset of harvest with the application of 0.29 mM and 0.29% in spring and 1.71 mM and 1.71% in summer, respectively.

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Conflict of Interest

The authors have no conflict of interest

Authors' Contribution

Anderson Carlos de Melo Gonçalves: Experiment design, data collection, initial scientific writing, formatting, submission, and overseeing the publication process; José de Anchieta Alves de Albuquerque: Guidance and scientific writing review; Evandro Franklin de Mesquita and Thiago Jardelino Dias: Co-guidance and scientific writing review; Caio da Silva Sousa and Alex Serafim de Lima: Data collection and scientific writing review; Toshik Iarley da Silva: Statistical analysis, scientific writing review, and translation into English; Aloisio Alcantara Vilarinho, José Maria Arcanjo Alves, Leandro Torres de Souza and Walter Esfrain Pereira: scientific writing review.

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E-mail and ORCID

Anderson Carlos de Melo Gonçalves (Corresponding author, anderson.agroufpb@yahoo.com), ORCID: https://orcid.org/0000-0003-4151-1192
Caio da Silva Sousa (caiosilvafla16@gmail.com), ORCID: https://orcid.org/0000-0002-4163-8313
Alex Serafim de Lima (alexcdf14@gmail.com), ORCID: https://orcid.org/0000-0002-8996-9592
Toshik Iarley da Silva (iarley.toshik@gmail.com), ORCID: https://orcid.org/0000-0003-0704-2046
José de Anchieta Alves de Albuquerque (anchietaufrr@gmail.com), ORCID: https://orcid.org/0000-0003-4391-258X
Evandro Franklin de Mesquita (evandrofranklin@servidor.uepb.edu.br), ORCID: https://orcid.org/0000-0001-5722-2235
Thiago Jardelino Dias (thiagojardelinodias@gmail.com), ORCID: https://orcid.org/0000-0002-7843-6184
Walter Esfrain Pereira (walterufpb@yahoo.com.br), ORCID: https://orcid.org/0000-0003-1085-0191
José Maria Arcanjo Alves (arcanjo.alves@ufrr.br), ORCID: https://orcid.org/0000-0002-8790-6838
Leandro Torres de Souza (leandro.torres@ufrr.br), ORCID: https://orcid.org/0000-0002-6970-2671
Aloisio Alcantara Vilarinho (aloisio.vilarinho@embrapa.br), ORCID: https://orcid.org/0009-0004-4415-8691