

Foliar salicylic acid application to mitigate the effect of water deficiency on potato (*Solanum tuberosum* L.)

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ABSTRACT

Water deficiency (WD) is one of the main factors that limit the growth and productivity of crops in the world. Salicylic acid (SA) is considered a plant hormone that has an important role in the plants' growth, development, and tolerance to abiotic stress. There is a hypothesis that the application of SA could mitigate the impact of the physical stress in potato plants. From the imposition of WD the physiological and biochemical indexes, including the antioxidative defense system, were evaluated in potato plants treated with the foliar application of SA. The assay revealed that the moderate WD 45 kPa negatively affected the metabolic parameters in potato plants. Foliar application of SA presented positive effects in the mitigation of oxidative damage through the dismutation of reactive oxygen species (ROS), suggesting the protection of the cellular membrane structure. Furthermore, there was less accumulation of malondialdehyde and synthesis of bioactive compounds, which certainly increased the antioxidative capacity of the plants. In conclusion, the results indicate that the foliar application of SA can have a positive regulatory role in alleviate water deficiency stress in potato plants.

1. Introduction

Abiotic stress is caused by any change in the environmental conditions where the plant is growing and can slow down its development. The main abiotic stresses include high and low temperatures, drought, flood, light, radiation, salinity, heavy metals or changes in the nutrient levels (Vats, 2018).

Water deficiency (WD) is an abiotic factor that negatively affects the growth and productivity of plants, altering morphological, biochemical, and molecular characteristics (Balestrini et al., 2018). A constant state of WD causes the reproductive development upsets, leading to the premature senescence of the leaves, withering, desiccation and death (Reddy et al., 2004).

Damage caused by WD are mainly due to the accumulation of reactive oxygen species (ROS). (Kundu et al., 2020). ROS can trigger harmful effects, such as damage to the DNA structure, amino acids and protein oxidation and lipidic peroxidation. In this last process, the phospholipid

components of the cellular membranes are oxidized, which alters their biological function, causing loss of fluidity and inactivation of enzymes linked to the membrane (Gill and Tuteja, 2010).

Phenolic compounds are an important class of plant secondary metabolites with a wide variety of medical and pharmacological uses. Biosynthesis of the phenolic compounds is triggered as a response to different biotic and abiotic stresses, as a defense mechanism, specially regarding its antioxidant capacity, which neutralizes reactive oxygen species (ROS) created by several stress factors (Khalil et al., 2018).

In addition to the plant immunological signaling, salicylic acid (SA) also acts in the signaling of several physiological responses, such as drought, thermogenesis, stomatal closing, seed germination, flowering, salt stress, ozone and cooling (Kumar, 2014; Wani et al., 2017).

Salicylic acid acts as an important regulator of plant growth, in the modulation of physiological and biochemical characteristics under abiotic stress. The hormone reduces the stress by increasing the proline, antioxidants, heat shock proteins (HSPs), secondary metabolites and

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sugars responses. SA favors plant tolerance mechanisms against physical stresses, such as salt, heavy metals, drought, cold, heat and UV radiation (Arif et al., 2020).

Potato is a typical temperate climate crop, sensitive to water deficit (Lizana et al., 2021). However, large part of the crop planting area in Brazil and in the world presents a lack of water resources, this factor being able to influence the growth and productivity of the potato.

Magnitude of the drought effects in the potato production depends on the phenological moment (sprouting, vegetative growth, tuberization and tuber bulk), duration and severity of the stress. Sprouting and tuberization are two critical phases when the WD most affects the final production of the potato. Drought reduces plant growth, shortens the growth cycle and reduces the number and size of the tubers. Water limitation reduces leaf growth, leaf area index, photosynthesis rate per leaf area unit ($\mu\text{CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and harvest index. The decline in photosynthesis rate is fast and substantial, even in relatively high-water potentials, in the range from -0.3 to -0.5 MPa (Monneveux et al., 2013). By reducing the transpiration rates, WD can cause an increase in plant temperature that, in turn, is harmful for the formation of potatoes (Bustan et al., 2004).

The aim of the assay was to determine if the foliar application of SA can alleviate the effects of oxidative stress induced by WD. Gas exchange parameters, antioxidant enzymes, bioactive compounds, variables linked to photosynthetic productivity and anatomic responses in potatoes under WD were evaluated in order to confirm the main hypotheses.

2. Material and methods

2.1. Plant material and treatments

The experiment was conducted in a plastic tunnel greenhouse, in pots with capacity for 50 L of soil. Pluvial precipitation, irrigation and maximum and minimum temperatures were recorded during the experiments.

Soil samples were collected from the 0.0–0.20 m depth layer, to determine the nutrient content (van Raij et al., 2001), and granulometric characteristics (Embrapa, 2013).

Water retention curve was adjusted using the method proposed by van Genuchten (1980). The water tensions corresponding to the depths used in the experiment were determined through the soil water retention curve. This curve was determined in the Soil and Natural Resources Department at UNESP-FCA. Thus, undisturbed soil samples were collected from 0 – 20 cm depth, which were subjected to the pressure chamber method (Richards, 1949; Topp and Zebchuk, 1979).

Potato cultivar used was cv Agata (Multiplant Vegetable Technology), type mini potato seed tuber, produced in laboratory through the process of meristem culture, micropropagation and acclimatization in protected environment, basic category G0; tuber caliber ≥ 2 cm diameter. With a 90 – 100-day cycle, depending mainly on the sowing time and climate. They were planted at a 12 cm depth, using one seed tuber per pot with a diameter between 30 and 50 mm, each containing vigorous shoots.

Soil matric potential was monitored with tensiometers (13 mm in diameter with ceramic porous cup connected with tubing to a mercury manometer). Tensiometers were installed 15 cm from the plant and the center of the porous capsule was 20 cm deep, with four (4) tensiometers per treatment, making a total of 16 monitoring points for the soil water tension, considering the two studied water depths. The readings were performed daily with the aid of a digital tensiometer.

The experimental outline was in randomized blocks (RDB), in a 2×2 factorial scheme, with 5 repetitions. The treatments consisted of control (with no water stress – 10 kPa) and severe water stress (45 kPa) and two levels of foliar application of salicylic acid (no application and 1.0 mM) (Table 1). Each vase with a single plant represented an experimental unit (one replication of a single treatment).

Table 1
Experimental treatments.

Treatments	Description
Control (CK)	10 kPa swt
Control (CK) + SA	10 kPa swt + foliar application in the leaf of 1 mM SA
WDS	45 kPa swt
WDS + SA	45 kPa swt + foliar application in the leaf of 1 mM SA

Abbreviation: [swt]: soil water tension, [WDS]: water deficit stress, [SA]: salicylic acid.

At 60 days after emergence (DAE), five repetitions were used for destructive evaluations for leaf and tuber. Five remaining repetitions were cultivated until the end of the experiment (90 DAE).

Foliar application of salicylic acid through spraying began at 30 DAE and ended at 90 DAE with an application interval of 48 h. On the same dates of foliar application of salicylic acid, deionized water was sprayed on the plants from the other treatments.

In the treatments with water deficiency, the soil water tension of 45 kPa was established at 30 DAE and maintained until 90 DAE. During this period of 60 days from tuber growth until tuber bulking, the pots were watered only when the soil matric potential reached the established potentials.

2.2. Chlorophyll and carotenoids content

Extraction was conducted from 0.025 g of frozen and grounded leaf. The material was weighed in 2 mL Eppendorf tubes and the pigment extraction was performed adding 2 mL of cold acetone (80%), according to Lichtenthaler and Buschmann (2001). Total chlorophyll and carotenoids were measured spectrophotometrically at 663 and 646 nm, respectively.

2.3. Photosynthetic parameters

Gas exchange parameters were determined on the third part of completely expanded leaves between 10:00 and 11:00 in the morning, using a portable infrared gas analyzer photosynthetic system (LI-COR 6400, LI-COR, Lincoln, NE, USA). In order to measure the liquid photosynthetic rate (Pn), stomatal conductance (gs), internal CO_2 concentration (Ci), transpiration rate (E); the air temperature, relative humidity, CO_2 concentration and PPFD (photosynthetic photon flux density) were kept at 25 °C, 85%, 380 $\mu\text{mol mol}^{-1}$ and 800 $\mu\text{mol (photon) m}^{-2} \text{ s}^{-1}$, respectively, as described by Zhang et al. (2009). At least six measures of the gas exchange components were conducted.

2.4. Mineral nutrients content

To determine the N, P, K and Mg content, the methodology described by Malavolta et al. (1997) was used. The total amounts of extracted nutrients were obtained by multiplying the nutrient content by the amount of dry matter accumulated in each part of the plant (leaf and tuber) and then adding them.

2.5. Malondialdehyde (MDA) content

MDA content, 1 g of leaf and tuber were mixed with 5 mL of 0.1% trichloroacetic acid solution and ground in low temperature. The homogenate was added to 5 mL of thiobarbituric acid (0.5%) and kept in boiling hot water bath for 15 min. The mixture was put in an ice bath to cool down to room temperature and then centrifuged. The absorbance of the supernatant was measured at 600 nm, 532 nm and 450 nm to calculate the MDA content (Wu et al., 2014).

2.6. Determining the total phenolic and flavonoid content and antioxidant activity

2.6.1. Total phenolic content (TPC)

Total phenolic compounds content was determined according to Lachman et al. (2008), using the Folin-Ciocalteu reagent. The extraction of phenolic compounds was conducted from frozen samples (2 g) with methanol solution (80%) for 24 h. Initially, the samples were sonicated for 15 min and agitated for 1 h at room temperature. The sample was filtered, and the volume was adjusted to 100 mL. Then, 5 mL of Folin-Ciocalteu reagent was added to 5 mL of sample, after agitation followed by the addition of 7.5 mL of sodium carbonate solution at 20%.

2.6.2. Total flavonoids (TF)

Total flavonoid content was determined according to Dewanto et al. (2002). The test sample (1 mL) was put in a 10 mL volumetric flask. Ethanol (60%, 8 mL) was added followed by 5% NaNO₂ (0.2 mL). After 6 min, AlCl₃ (10%, 0.2 mL) was added. After another 6 min, NaOH (4%, 0.6 mL) was added and then, water to complete 10 mL. The solution was mixed, and the absorbency was measured at 415 nm. The measures were conducted in triplicate and the calculations were based on a standard curve obtained with rutin p.a. Total flavonoid content was expressed in micrograms of rutin equivalents per milligram of sample.

2.6.3. Ferric reducing antioxidant power (FRAP)

The reduction of antioxidants in the ferric-tripyridyl triazine (Fe³⁺-TPTZ) to the ferrous form (Fe²⁺), determined according to Benzie and Strain (1996). The FRAP reagent was freshly prepared mixing acetate buffer (100 mL, 300 mM, pH 3.6), TPTZ solution (10 mL of TPTZ, 10 mM in 40 mM / HCl), FeCl₃ 6H₂O (10 mL, 20 mM) in a proportion of 10:1:1 and addition of distilled water (10 mL), at 37 °C. To conduct the assay, FRAP reagent (1.8 mL), deionized water (180 µL) and sample, standard or white (60 µL) were then added to the test tube and incubated at 37 °C for 4 min; the absorbency was measured at 593 nm, using the FRAP work solution as white. In the FRAP assay, the antioxidant potential of the sample was determined from a standard curve outlined using the linear regression equation FeSO₄ • 7H₂O to calculate the FRAP values of the sample.

2.6.4. 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

Spectrophotometric analysis of the ABTS⁺ radical scavenging activity was determined using the method by Re et al. (1999). ABTS⁺ cation radical was produced by the reaction between ABTS (7 mM) in H₂O and potassium persulfate (2.45 mM), stored in the dark at room temperature for 24 h. Before use, the ABTS^{•+} solution was diluted to an absorbency of 0.70 ± 0.02 at 732 nm with phosphate buffer (0.1 M, pH 7.4). ABTS⁺ solution (990 µL) was added to the sample (10 µL). After 1 min, an inhibition percentage of 732 nm was calculated for each concentration in relation to an absorbance in blank.

2.7. Antioxidant enzyme extraction

For antioxidant enzyme activities, 1 g of leaf powder was homogenized in 1.5 mL of extraction buffer containing 50 mmol·L⁻¹ of sodium phosphate buffer (pH 7.6), 10 mmol·L⁻¹ of sodium metabisulfite, 1 mmol·L⁻¹ of ascorbic acid, 1 mmol·L⁻¹ of ethylenediamine tetra acetic acid (EDTA), 20% (p/v) sorbitol and 2% (p/v) polyvinyl polypyrrolidone (PVPP) and centrifuged at 12 000 x g for 20 min at 4 °C Bajji et al. (2007). Protein content were determined according to Lowry et al. (1951). Enzyme activities were measured immediately in fresh flasks.

2.7.1. Superoxide dismutase (SOD) activity

SOD activity was measured based on the inhibition of NBT - nitroblue tetrazolium photoreduction in the presence of riboflavin (Dhindsa et al., 1981). One unit of SOD activity was defined as the amount of enzyme

necessary to inhibit 50% of NBT reduction in 1 min in the presence of riboflavin and light, monitored at 560 nm and expressed as specific activity in units per mg of protein (U mg⁻¹ protein).

2.7.2. Peroxidase (POD) activity

Peroxidase (POD, EC 1.11.1.7) activity was determined by the addition of 100 µL of crude extract, diluted in the proportion of 1:25 (v/v), for 4.9 mL of a solution containing potassium phosphate 25 mM buffer, pH 6.8, pyrogallol 20 mM and H₂O₂ 20 mM. After incubating the solution at 250 °C for 1 min, the reaction was interrupted by the addition of 0.5 mL of H₂SO₄ at 5% (v/v) and the absorbency measured at 420 nm. Enzyme activity was calculated using a molar extinction coefficient of 2.47 mM⁻¹ cm⁻¹ (Peixoto et al., 1999).

2.8. Proline accumulation

Proline content in leaves samples was determined as described by Bates et al. (1973). The proline was extracted in sulfosalicylic acid at 3%. In the extract, an equal volume of glacial acetic acid and ninhydrin solutions were added. The sample was heated at 100 °C, to which 5 mL of toluene were added after cooling. The absorbance of the toluene layer was measured at 520 nm, in spectrophotometer.

2.9. Statistical analysis

All data was obtained through five repetitions per evaluation. The statistical analysis was conducted using the statistical software Minitab 17 through unilateral variance analysis. The Tukey test (*P* < 0.05) was applied to the statistical analysis of significant differences between the irrigation depths and application of salicylic acid. The principal components analysis (PCA) was applied to all physiological, mineral and biochemical attributes.

3. Results

ANOVA results for soil water tensions and foliar application of salicylic acid and their interactions in the photosynthetic, biochemical parameters and nutrient concentration in the leaf and tuber are shown in Tables 2 and 3.

3.1. Chlorophyll and total carotenoids content

Results revealed a reduction in chlorophyll content in the control plant (1.21 mg g⁻¹) at 30 DAE and several other treatments at 60 DAE (Fig. 1A). At 60 DAE, highest chlorophyll content (0.74 mg g⁻¹) was recorded in plant form the control treatment with no foliar application of salicylic acid. In general, the water deficiency caused a reduction in the chlorophyll content in the plants' leaves.

Foliar application of salicylic acid and increase in soil water tension caused changes in the carotenoids content between 30 and 60 DAE. It was observed that the control treatment reverted the negative impact of the water deficiency stress on the carotenoids content at 60 DAE. However, foliar application of salicylic acid was not able to alleviate the negative effect (Fig. 1B).

3.2. Photosynthetic parameters

Gas exchanges are summarized in Fig. 2A, B. Pn and gs values were recorded in 25.20 µmol m⁻² s⁻¹ and 0.512 mol m⁻² s⁻¹, respectively, in the initial tuber phase of the potato plant at 30 DAE. At 30 and 60 DAE, they reduced significantly. Pn and gs showed a tendency to reduce in control plants with salicylic acid application, with values of 11.22 µmol m⁻² s⁻¹ and 0.164 mol m⁻² s⁻¹.

Transpiration rates (E) presented significant change between 30 and 60 DAE (Fig. 2C). It was observed that the control treatment with salicylic acid application presents higher transpiration rate values, 2.94

Table 2

ANOVA of soil water tensions (10 and 45 kPa), foliar application of SA (with and without SA) and their interactions on photosynthetic, biochemical and mineral parameters in potato leaf.

		Effect	dF	F-value	p-value
Chlorophyll	60 DAE	SWT	1	1.89ns	0.1945
		SA	1	0.81ns	0.3857
		SWT x SA	1	0.00ns	0.9453
Total Carotenoids	60 DAE	SWT	1	1.23ns	0.2885
		SA	1	1.68ns	0.2188
		SWT x SA	1	0.10ns	0.7577
MDA	60 DAE	SWT	1	1.40ns	0.2598
		SA	1	0.04ns	0.8512
		SWT x SA	1	1.84ns	0.1995
Total Phenolic Compounds	60 DAE	SWT	1	0.75ns	0.4023
		SA	1	0.33ns	0.5772
		SWT x SA	1	0.26ns	0.6179
Total Flavonoids	60 DAE	SWT	1	0.12ns	0.7316
		SA	1	0.04ns	0.8519
		SWT x SA	1	0.04ns	0.8441
FRAP	60 DAE	SWT	1	0.08ns	0.7779
		SA	1	0.40ns	0.5374
		SWT x SA	1	0.42ns	0.5312
ABTS	60 DAE	SWT	1	1.95ns	0.1877
		SA	1	4.64ns	0.0523
		SWT x SA	1	0.00ns	0.9719
N	60 DAE	SWT	1	1074.41**	<0.0001
		SA	1	418.42**	<0.0001
		SWT x SA	1	42.90**	<0.0001
P	60 DAE	SWT	1	115.25**	<0.0001
		SA	1	25.35**	<0.0001
		SWT x SA	1	77.57**	<0.0001
K	60 DAE	SWT	1	1847.59**	<0.0001
		SA	1	17.56**	0.0013
		SWT x SA	1	4.69ns	0.0512
Mg	60 DAE	SWT	1	28.03**	0.0002
		SA	1	160.40**	<0.0001
		SWT x SA	1	49.83**	<0.0001
Pn	60 DAE	SWT	1	12.82**	0.0038
		SA	1	0.03ns	0.8606
		SWT x SA	1	1.03ns	0.3311
E	60 DAE	SWT	1	32.00**	<0.0001
		SA	1	0.98ns	0.3424
		SWT x SA	1	1.31ns	0.2742
gs	60 DAE	SWT	1	31.10*	<0.0001
		SA	1	0.93ns	0.3536
		SWT x SA	1	1.06ns	0.3237
Ci	60 DAE	SWT	1	3.29ns	0.0948
		SA	1	0.45ns	0.5131
		SWT x SA	1	0.27ns	0.6132

mmol m⁻² s⁻¹, in comparison to the plant in water deficiency conditions with salicylic acid application, which presented values of 1.41 mmol m⁻² s⁻¹.

Internal CO₂ concentration slightly reduced in the different treatments at 60 DAE (Fig. 2D). This became evident through the reduction observed in the value of 285.59 μmol m⁻² s⁻¹ in the control plant at 30 DAE to 214.46 μmol m⁻² s⁻¹ in plants in water deficiency condition with no salicylic acid spraying.

3.3. Mineral nutrients content

N content in the leaf reduced between 30 and 60 DAE in all treated plants in comparison to the control plant. At 60 DAE, a significant reduction in N concentration was observed in the leaf of the plant in water deficiency condition with no salicylic acid application (WDS) in comparison to the control plants with salicylic acid application (CK+SA) which presented higher N concentration values (Table 4).

For the tuber, the N level increased for the plants (WDS+SA) compared to the plants in water deficit conditions with no salicylic acid application (WDS), which evidenced the lowest values of N content at 90 DAE (Table 5).

Salicylic acid application and soil water retention of 10 kPa resulted

Table 3

ANOVA of soil water tensions (10 and 45 kPa), foliar application of SA (with and without SA) and their interactions on photosynthetic, biochemical and mineral parameters in potato tuber.

		Effect	dF	F-value	p-value
Total Phenolic Compounds	60 DAE	SWT	1	1.74ns	0.2116
		SA	1	0.33ns	0.5765
		SWT x SA	1	0.01ns	0.9138
	90 DAE	SWT	1	0.86ns	0.3724
		SA	1	0.02ns	0.9045
		SWT x SA	1	1.19ns	0.2970
Total Flavonoids	60 DAE	SWT	1	2.14ns	0.1691
		SA	1	0.00ns	0.9466
		SWT x SA	1	1.53ns	0.2397
	90 DAE	SWT	1	0.16ns	0.6997
		SA	1	0.07ns	0.8009
		SWT x SA	1	0.07ns	0.7973
FRAP	60 DAE	SWT	1	0.29ns	0.6004
		SA	1	0.36ns	0.5597
		SWT x SA	1	0.37ns	0.5526
	90 DAE	SWT	1	0.52ns	0.4841
		SA	1	3.81ns	0.0748
		SWT x SA	1	10.49**	0.0071
ABTS	60 DAE	SWT	1	5.25*	0.0409
		SA	1	0.27ns	0.6116
		SWT x SA	1	2.62ns	0.1315
	90 DAE	SWT	1	3.29ns	0.0949
		SA	1	1.46ns	0.2507
		SWT x SA	1	4.10ns	0.0656
MDA	60 DAE	SWT	1	0.21ns	0.6518
		SA	1	2.27ns	0.1577
		SWT x SA	1	0.70ns	0.4205
	90 DAE	SWT	1	0.05ns	0.8269
		SA	1	0.18ns	0.6817
		SWT x SA	1	2.65ns	0.1305
N	60 DAE	SWT	1	196.93**	<0.0001
		SA	1	1.09ns	0.3163
		SWT x SA	1	22.03**	0.0005
	90 DAE	SWT	1	44.21**	<0.0001
		SA	1	64.24**	<0.0001
		SWT x SA	1	150.99**	<0.0001
P	60 DAE	SWT	1	4.78*	0.0494
		SA	1	204.78**	<0.0001
		SWT x SA	1	285.17**	<0.0001
	90 DAE	SWT	1	187.86**	<0.0001
		SA	1	0.13ns	0.7230
		SWT x SA	1	8.37*	0.0135
K	60 DAE	SWT	1	755.22**	<0.0001
		SA	1	922.58**	<0.0001
		SWT x SA	1	2736.41**	<0.0001
	90 DAE	SWT	1	0.49ns	0.4991
		SA	1	24.85**	0.0003
		SWT x SA	1	0.14ns	0.7140
Mg	60 DAE	SWT	1	1.52ns	0.2407
		SA	1	0.38ns	0.5486
		SWT x SA	1	13.71**	0.0030
	90 DAE	SWT	1	1.21ns	0.2929
		SA	1	0.62ns	0.4473
		SWT x SA	1	0.62ns	0.4473

in a higher P content in the leaves. P content was 2.04 g kg⁻¹ in CK+SA plants to 1.41 g kg⁻¹ in plants in water deficit conditions with no salicylic acid application (WDS), a difference of approximately 1.44 times in the P content in the potato plant leaf at 60 DAE (Table 4).

Highest P content in tuber at 90 DAE (2.72 g kg⁻¹) was found in control plants with no salicylic acid application (CK), however, without presenting significant difference in control plants with salicylic acid application (CK-SA) (2.64 g kg⁻¹) (Table 5).

K content in the plant leaf significantly increased in treatments (CK) and (CK+SA) at 60 DAE. It was observed that the soil water tension (10 kPa) induced an increase in potassium content in comparison to the treatments in water deficiency conditions (Table 4). For the tubers at 90 DAE, it was evidenced that the treatments (CK and WDS) presented higher K contents (28.42 and 27.82 g kg⁻¹), respectively, in comparison

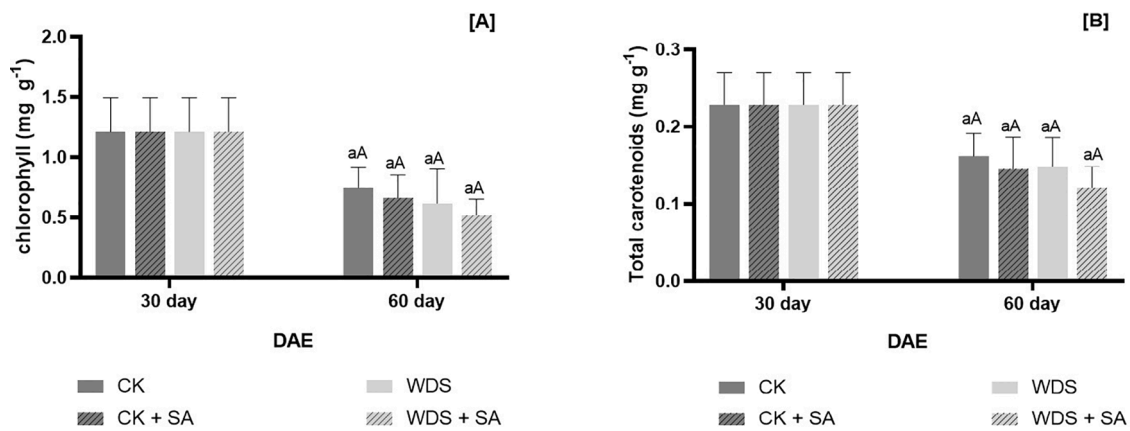


Fig. 1. Effect of foliar application of salicylic acid (SA) and soil water tension on photosynthetic pigments, chlorophyll content (A) and total carotenoids (B) in leaves of potato plant. Different capital letters between SA concentrations (with and without SA under equal water conditions) and lower case letters between water conditions (10 and 45 kPa under equal SA conditions) indicate significant differences from the Tukey test ($p \leq 0.05$). Data corresponding to means of five repetitions.

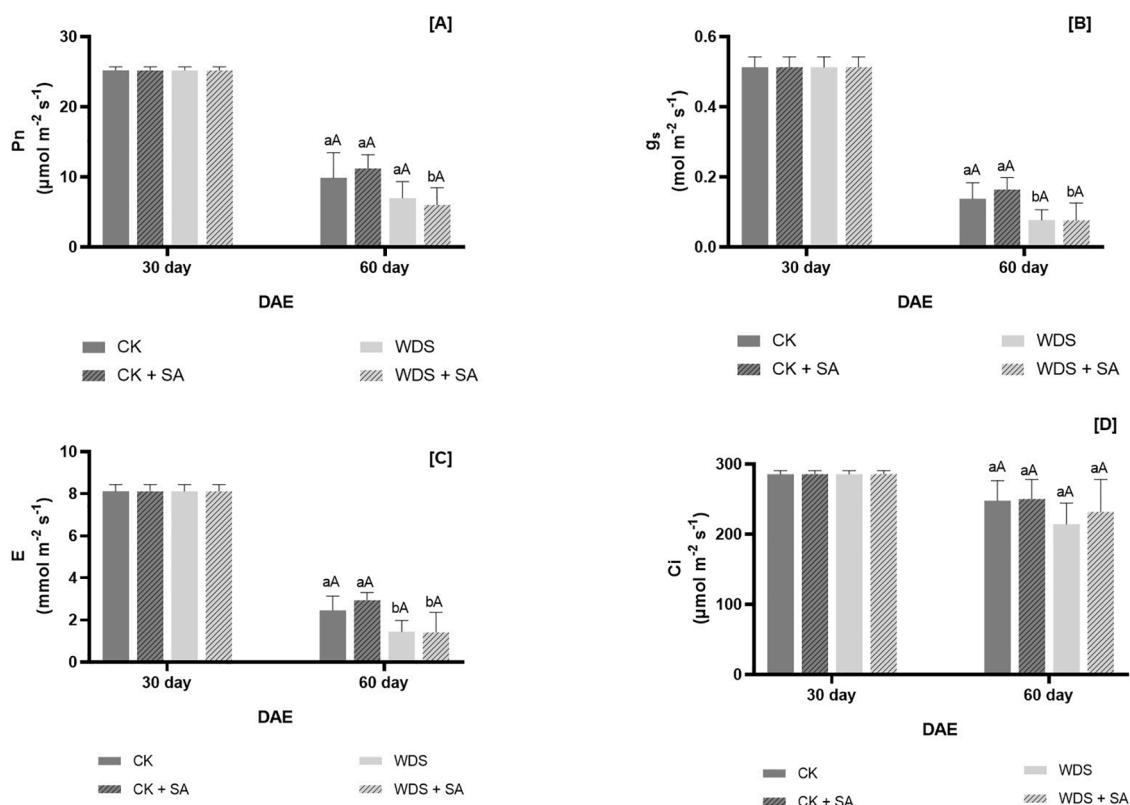


Fig. 2. Effect of foliar application of salicylic acid (SA) and soil water tension on photosynthetic gas exchange parameters, photosynthetic rate (Pn) (A), stomatal conductance (g_s) (B), transpiration rate (E) (C) and internal CO_2 concentration (Ci) (D) in leaves of potato plant. Different capital letters between SA concentrations (with and without SA under equal water conditions) and lower case letters between water conditions (10 and 45 kPa under equal SA conditions) indicate significant differences from the Tukey test ($p \leq 0.05$). Data corresponding to means of five repetitions.

to the other treatments (Table 5).

Results revealed that there is significant difference between the Mg content in the leaves of CK+SA and WDS+SA plants in comparison to the other treatments that did not have foliar application of salicylic acid, as summarized in Table 4. Mg content in the tubers did not present significant difference at 90 DAE.

3.4. MDA content

MDA content, an indicator of lipidic peroxidation, was more pronounced in plants kept under water deficit stress with salicylic acid

application ($4.72 \mu\text{mol g}^{-1}$) when compared to control plants with salicylic acid application ($3.35 \mu\text{mol g}^{-1}$) at 60 DAE (Fig. 3A). The results revealed that there is difference in the MDA content in the tuber in all treatments between 60 and 90 DAE. In the control treatment with salicylic acid application, the tubers presented a lower MDA content ($1.70 \mu\text{mol g}^{-1}$) in relation to the plants with water deficit stress with salicylic acid application ($2.02 \mu\text{mol g}^{-1}$) at 90 DAE (Fig. 3B).

3.5. TPC and TF content

Forleaves, the application of salicylic acid in WD conditions

Table 4

Macronutrient concentration in potato plant leaves on foliar application of salicylic acid (SA) and soil water tension (SWT).

DAE	SWT (kPa)	AS	N (g kg ⁻¹)	P (g kg ⁻¹)	K (g kg ⁻¹)	Mg (g kg ⁻¹)
30		-	33.13	4.6	57.56	4.6
60	10	SA	±0.14 ^{ab}	±0.04 ^{ab}	±0.31 ^{aA}	±0.14 ^{ab}
		+	31.06	2.04	71.40	6.26
		SA	±0.27 ^{aA}	±0.02 ^{aA}	±0.57 ^{aA}	±0.10 ^{aA}
	45	-	19.96	1.53	51.36	2.80
		SA	±0.09 ^{bB}	±0.01 ^{aA}	±0.27 ^{bB}	±0.06 ^{bB}
		+	22.86	1.41	54.06	6.60
		SA	±0.15 ^{bA}	±0.02 ^{bB}	±0.18 ^{bA}	±0.27 ^{aA}
Effect	dF					
SWT	1		**	**	**	**
SA	1		**	**	**	**
SWT x SA	1		**	**	ns	**
CV			1.87	4.39	1.53	8.26

Mean values ± SE (n = 5). Different capital letters between SA concentrations (with and without SA under equal water conditions) and lower case letters between water conditions (10 and 45 kPa under equal SA conditions) indicate significant differences from the Tukey test.

* Significance at 0.05 level.

** Significance at 0.01 level.

Table 5

Macronutrient concentration in potato tubers under foliar application of salicylic acid (SA) and soil water tension (SWT).

DAE	SWT (kPa)	SA	N (g kg ⁻¹)	P (g kg ⁻¹)	K (g kg ⁻¹)	Mg (g kg ⁻¹)
60	10	-	14.02	1.69	27.74	1.44
		SA	±0.17 ^{bb}	±0.04 ^{bb}	±0.08 ^{aA}	±0.02 ^{aA}
		+	14.47	2.76	20.88	1.30
	45	SA	±0.06 ^{bA}	±0.00 ^{aA}	±0.05 ^{bb}	±0.01 ^{bb}
		-	16.33	2.34	25.68	1.36
		SA	±0.08 ^{aA}	±0.01 ^{aA}	±0.07 ^{bb}	±0.04 ^{ab}
90	10	+	15.62	2.25	27.50	1.46
		SA	±0.04 ^{ab}	±0.03 ^{ba}	±0.06 ^{aA}	±0.02 ^{aA}
		-	14.81	2.72	28.42	1.38
	45	SA	±0.14 ^{aA}	±0.02 ^{aA}	±0.31 ^{aA}	±0.06 ^{aA}
		+	13.88	2.64	25.42	1.28
		SA	±0.26 ^{bb}	±0.04 ^{aA}	±0.78 ^{ab}	±0.05 ^{aA}
90	45	-	13.58	2.17	27.82	1.40
		SA	±0.10 ^{bb}	±0.01 ^{bb}	±0.33 ^{aA}	±0.06 ^{aA}
		+	17.98	2.28	25.24	1.40
		SA	±0.20 ^{aA}	±0.01 ^{bA}	±0.31 ^{ab}	±0.02 ^{aA}
Effect	dF					
SWT (60 DAE)	1		**	*	**	ns
SA (60 DAE)	1		ns	**	**	ns
SWTxSA (60DAE)	1		**	**	**	**
CV (60DAE)			1,82	3,39	0,72	5,21
SWT (90 DAE)	1		**	**	ns	ns
SA(90 DAE)	1		**	ns	**	ns
SWTxSA (90DAE)	1		**	*	ns	ns
CV (90 DAE)			3,21	3,00	4,68	10,42

Mean values ± SE (n = 5). Different capital letters between SA concentrations (with and without SA under equal water conditions) and lower case letters between water conditions (10 and 45 kPa under equal SA conditions) indicate significant differences from the Tukey test.

* Significance at 0.05 level.

** Significance at 0.01 level.

promoted the increase of total phenolic compounds 2.93 µg GAE mg⁻¹ FW at 60 DAE (Fig. 4A). In tubers at 90 DAE, there was also a slight increase in phenolic compounds in all treatments CK, CK+SA, WDS and WDS+SA (0.30, 0.32, 0.34 and 0.31 µg GAE mg⁻¹ FW, respectively), but it was not significant when compared to tubers at 60 DAE (Fig. 4B).

For the total flavonoids content, in potato leaves, there was significant increase for all treatments between 30 and 60 DAE (Fig. 5A). The CK and CK+SA plants presented higher flavonoids content (7.31 and 7,30 µg rutin mg⁻¹ FW, respectively) at 60 DAE. Tubers evidence a slight increase between 60 and 90 DAE in all treatments. A special behavior was observed in CK+SA tuber at 60 and 90 DAE, which presented change in flavonoids content from 0.36 to 0.49 µg rutin mg⁻¹ FW, respectively (Fig. 5B).

3.6. Antioxidant capacity

Water deficiency conditions induced (leaf and tuber) an increase in the values of FRAP and ABTS antioxidant tests. However, at 60 DAE, the leaves of WDS+SA potato plants reached the highest values in comparison to the control treatments at 30 DAE in FRAP and ABTS tests (Fig. 6A – C).

Application of salicylic acid in water deficit conditions resulted in reduction of the FRAP antioxidant proprieties of tubers at 90 DAE (Fig. 6B). Decrease in antioxidant activity (Fig. 6D), measured by the ABTS method was found in tubers that were subjected to water deficit conditions with no salicylic acid application (2.61 µM trolox mg⁻¹ FW)

3.7. Antioxidant enzymes and proline

Plants with foliar application of salicylic acid presented higher SOD and POD activity in leaves in comparison to control plants.

Control plants with salicylic acid (CK+SA) presented higher increase in SOD activities (624.95 U g⁻¹ FW) in comparison to plants in water deficit with salicylic acid application that presented lower activity values –330.99 U g⁻¹ FW (Fig. 7A).

POD activity in all treatments increased considerably at 60 DAE in comparison to non-treated plants (CK) (1.66 U g⁻¹ FW) at 30 DAE.

Proline content increased significantly in all treatments between 30 and 60 DAE. The proline content increased in treatments CK+SA, WDS and WDS+SA, the highest proline content (2.66 µg g⁻¹ FW) was observed in WDS plants in comparison to control plants (CK) that presented lower values (1.72 µg g⁻¹ FW) at 60 DAE (Fig. 8).

3.8. Principal components analysis

Principal components analysis (PCA) of the leaf at 60 DAE was conducted in the data set from the results for the 4 treatments, from the combination of two soil water tensions and two doses of salicylic acid application, for the 18 characteristics evaluated in the leaf of the potato plant. Total variability was explained by 3 principal components (PCs). The first two (PC1 and PC2) represented 83.50% of the total variation (Table 6 and Fig. 9).

PC1 was responsible for 58.2% of the total variation, being efficient in the separation of CK and CK+SA treatments from the other treatments (Fig. 10A). Charge exam (Fig. 10B) suggested that this separation is due to TF, Ci, N, SOD, E, K, P, gs, Pn and Chl. All these characteristics presented high positive charges (>0.70) in the PCA.

The scores and charges of PC1 suggested that the CK and CK+SA plants at 60 DAE presented higher values for nitrogen, phosphorus, potassium content in the leaf, as well as higher values for photosynthetic rate, transpiration rate, internal CO₂ concentration and stomatal conductance. Opposite occurs in plants under water deficit conditions (WDS and WDS+SA), which presented higher values for total phenolic compounds and malondialdehyde.

PC2 was responsible for 25.3% of the total variation, being efficient mainly in WDS+SA plant (Fig. 10A). The examination of the PC2 charge

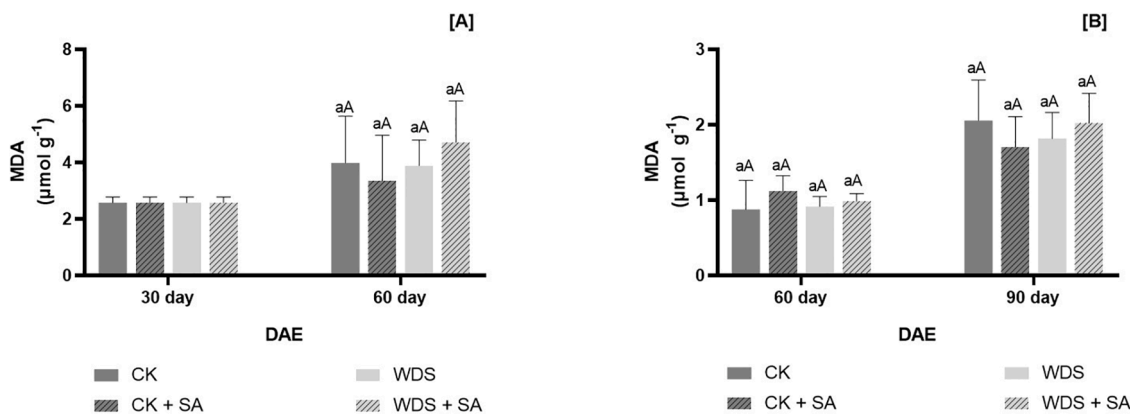


Fig. 3. Effect of foliar application of salicylic acid (SA) and soil water tension on malondialdehyde (MDA) content in leaf (A) and tuber (B) of potato plant. Different capital letters between SA concentrations (with and without SA under equal water conditions) and lower case letters between water conditions (10 and 45 kPa under equal SA conditions) indicate significant differences from the Tukey test ($p \leq 0.05$). Data corresponding to means of five repetitions.

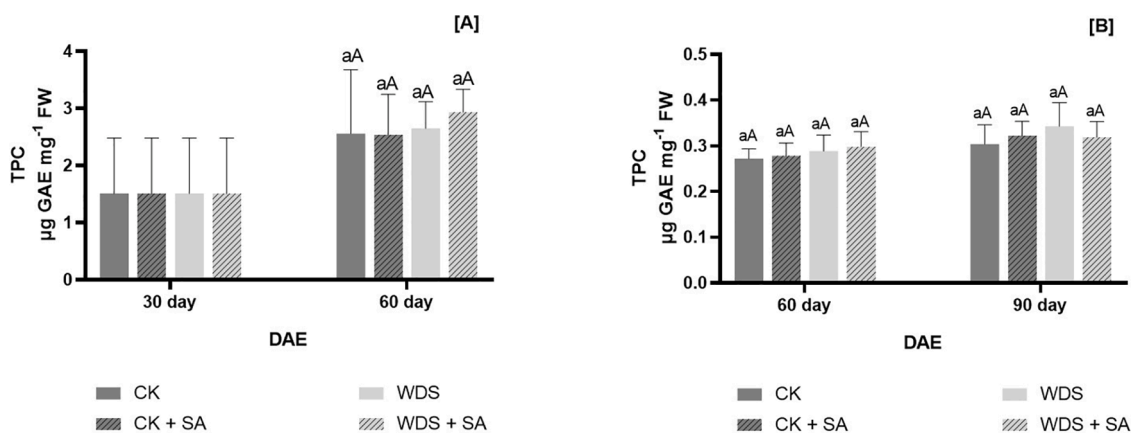


Fig. 4. Effect of foliar application of salicylic acid (SA) and soil water tension on total phenolic content (TPC) in leaf (A) and tuber (B) of potato plant. Different capital letters between SA concentrations (with and without SA under equal water conditions) and lower case letters between water conditions (10 and 45 kPa under equal SA conditions) indicate significant differences from the Tukey test ($p \leq 0.05$). Data corresponding to means of five repetitions.

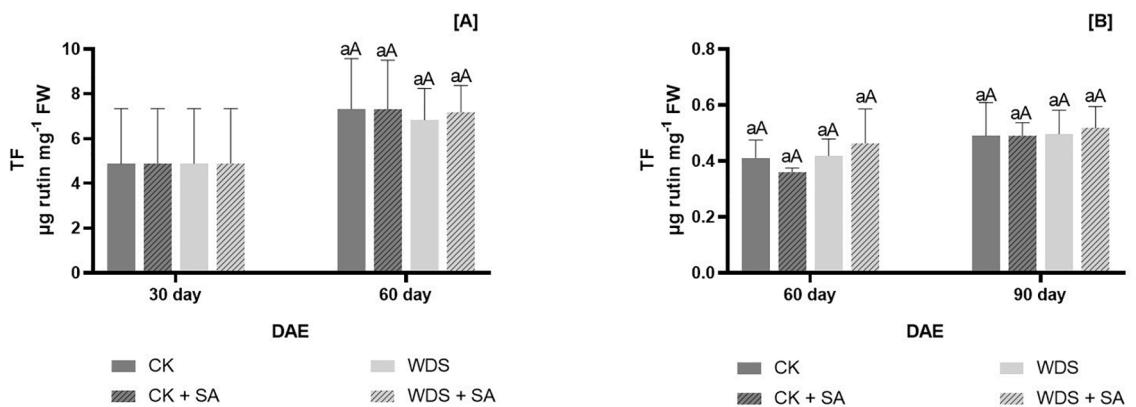


Fig. 5. Effect of foliar application of salicylic acid (SA) and soil water tension on total flavonoids (TF) in leaf (A) and tuber (B) of potato plant. Different capital letters between SA concentrations (with and without SA under equal water conditions) and lower case letters between water conditions (10 and 45 kPa under equal SA conditions) indicate significant differences from the Tukey test ($p \leq 0.05$). Data corresponding to means of five repetitions.

graphic (Fig. 10B) indicates that the most important variables that contributed to PC2 were the antioxidant test (FRAP and ABTS) and magnesium content, all with positive charges. PC2 scores and charges indicated that the plant under water deficiency with no salicylic acid application presents higher carotenoids values in the leaf.

Principal components analysis (PCA) of the tuber at 60 and 90 DAE

was conducted on the data set from the results of the 4 treatments, from the combination of two soil water tensions and two doses of salicylic acid application, for 10 characteristics evaluated in the tuber of the potato plant. Total variability was explained by 7 principal components (PCs). The first two (PC1 and PC2) represented 69.27% of the total variation (Table 7).

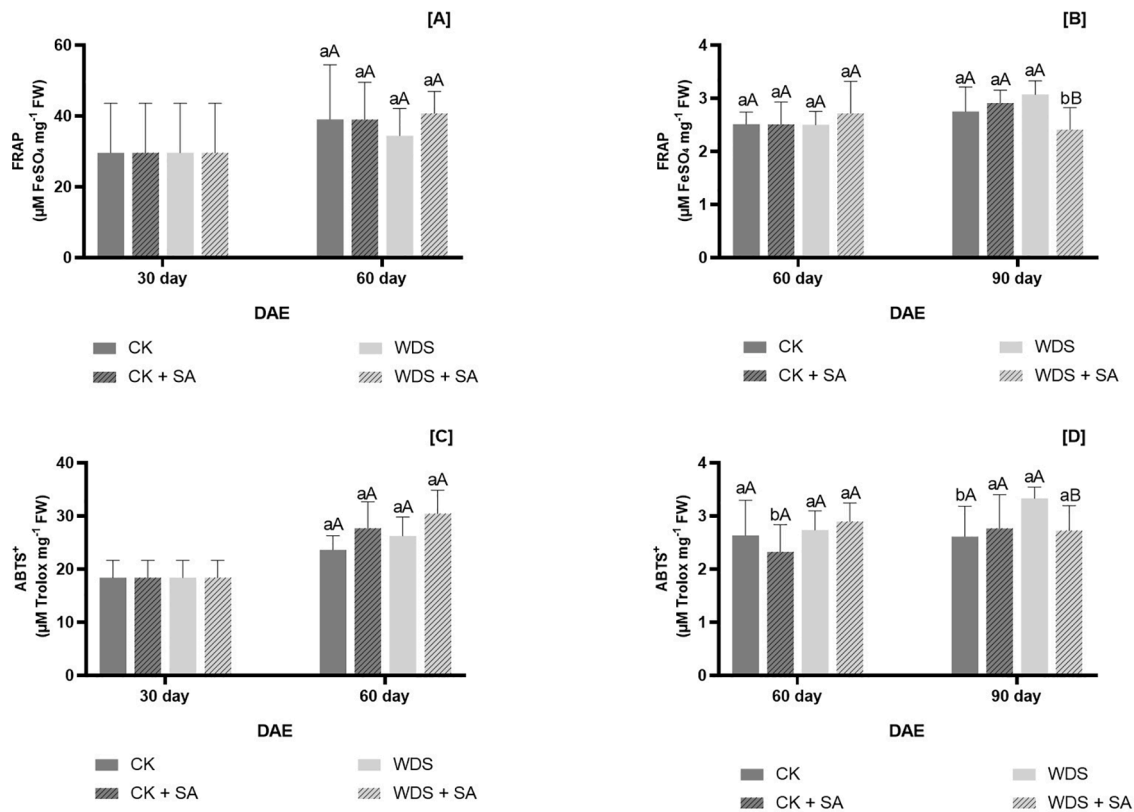


Fig. 6. Effect of foliar application of salicylic acid (SA) and soil water tension on antioxidant activity, FRAP activity of leaves (A), FRAP activity of tuber (B), ABTS⁺ activity of leaf (C) and ABTS⁺ activity of tuber (D) in the potato plant. Different capital letters between SA concentrations (with and without SA under equal water conditions) and lower case letters between water conditions (10 and 45 kPa under equal SA conditions) indicate significant differences from the Tukey test ($p \leq 0.05$). Data corresponding to means of five repetitions.

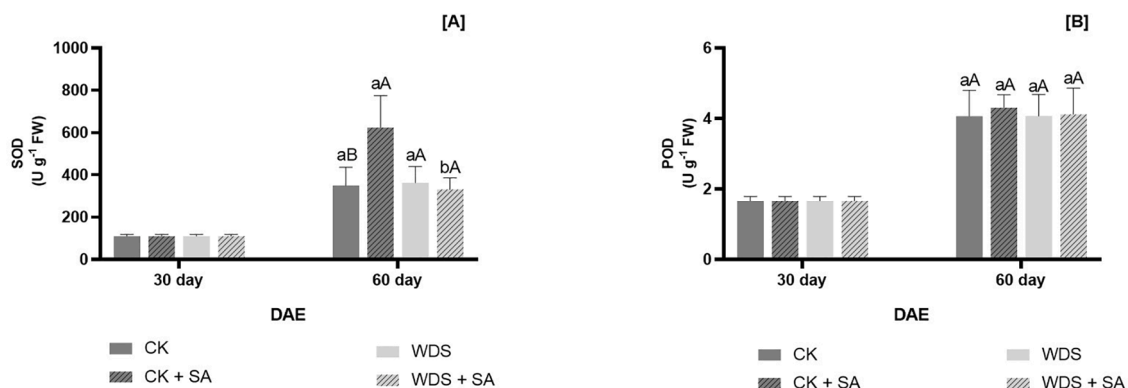


Fig. 7. Effect of foliar application of salicylic acid (SA) and soil water tension on the antioxidant enzyme, superoxide dismutase (SOD) (A) and peroxidase (POD) (B) of leaves in the potato plant. Different capital letters between SA concentrations (with and without SA under equal water conditions) and lower case letters between water conditions (10 and 45 kPa under equal SA conditions) indicate significant differences from the Tukey test ($p \leq 0.05$). Data corresponding to means of five repetitions.

PC1 was responsible for 42.2% of the total variation, being efficient in the separation of the WDS treatment at 90 DAE (Fig. 11A). The charge exam (Fig. 11B) suggested that this separation is due to TPC, TF, ABTS and K. All these characteristics presented high positive charges (>0.70) in the PCA.

PC1 scores and charges suggested that the tubers of the plant in water deficiency conditions with no foliar application of salicylic acid at 90 DAE presented higher values of phenolic compounds, flavonoids and antioxidant activity (ABTS).

PC2 was responsible for 27.1% of the total variation, being efficient mainly in the separation of CK+SA plant at 90 DAE (Fig. 11A). The

charge exam (Fig. 11B) suggested that this separation is due to the P content in the tuber. This characteristic presented high positive charges (>0.70) in the PCA.

4. Discussion

Negative effects of WD on the photosynthetic pigments are caused by the increase in chlorophyllase enzyme activity, which results in chlorophyll degradation, as well as the destruction of chloroplasts and instability of the protein complex caused by the increase in reactive oxygen species (ROS) levels (Askari and Ehsanzadeh, 2015).

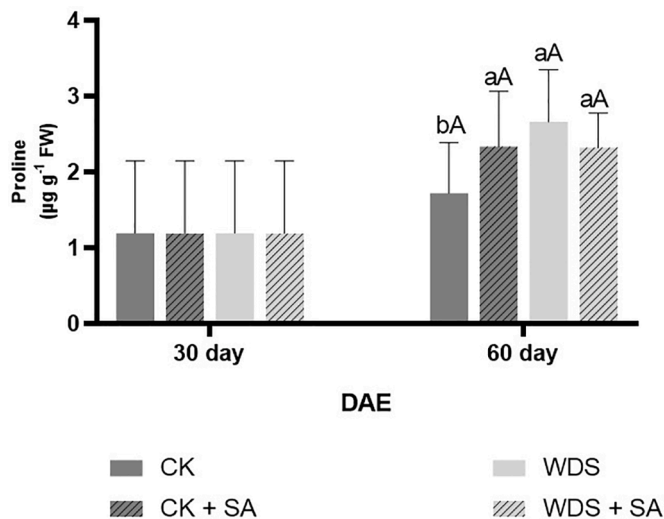


Fig. 8. Effect of foliar application of salicylic acid (SA) and soil water tension on the proline content of leaves in the potato plant. Different capital letters between SA concentrations (with and without SA under equal water conditions) and lower case letters between water conditions (10 and 45 kPa under equal SA conditions) indicate significant differences from the Tukey test ($p \leq 0.05$). Data corresponding to means of five repetitions.

Table 6

Factor loadings, eigenvalues and proportion of variation associated with three principal components (PC) of the PCA of 18 parameters in 4 treatments in potato leaf.

Characteristics	PC1	PC2	PC3
TPC	-0.764	0.635	-0.112
TF	0.748	0.511	-0.422
FRAP	0.275	0.857	-0.435
ABTS	-0.407	0.813	0.413
MDA	-0.751	0.434	-0.495
SOD	0.753	0.174	0.634
POD	0.643	0.458	0.613
Prol	-0.511	-0.043	0.858
Chl	0.738	-0.578	-0.343
Car	0.546	-0.795	-0.261
N	0.920	0.356	0.159
P	0.861	0.027	0.506
K	0.972	0.039	-0.227
Mg	0.374	0.907	-0.187
Pn	0.990	-0.128	0.052
E	0.999	0.011	0.026
gs	0.999	0.016	0.012
Ci	0.885	0.346	-0.309
Eigenvalue	10.47	4.55	2.96
Percent (%)	58.21	25.29	16.49
Accumulative (%)	58.21	83.50	100

Abbreviation: total phenolic compounds [TPC], total flavonoids [TF], malondialdehyde [MDA], superoxide dismutase [SOD], peroxidase [POD], proline [Prol], total chlorophyll [Chl], carotenoids [Car].

Furthermore, the reduction and later stability in chlorophyll a and b degradation suggests a reduction in the chlorophyll degradation process (Khapte et al., 2019), corroborating the hypothesis that SA mitigates the effects of WD. These results are in accordance with the findings by Lobato et al. (2021), who studied the exogenous application of salicylic acid under water deficit stress in tomatoes. In the current study, found that the foliar application of SA, in fact has a potential effect to minimize the pigments degradation. Li et al. (2019) also verified that the application of SA was able to positively promote the increase of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids levels in the potato crop under oxidative stress by cadmium in the soil, corroborating the results of the present study related to water deficiency.

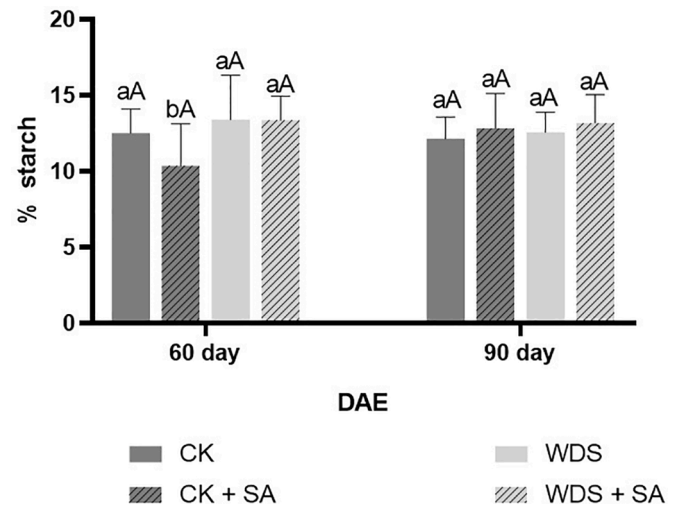


Fig. 9. Effect of foliar application of salicylic acid (SA) and water tension on % starch of tuber in the potato plant. Different capital letters between SA concentrations (with and without SA under equal water conditions) and lower case letters between water conditions (10 and 45 kPa under equal SA conditions) indicate significant differences from the Tukey test ($p \leq 0.05$). Data corresponding to means of five repetitions.

Exogenous application of SA probably reduced chlorophyllase activity combined with reduced ROS concentration and increase in carotenoids content. Carotenoids have additional functions in the dismutation of ROS, they stabilize photosynthetic complexes, participate in energy dissipation and they can also help plants to reduce the adverse effects of water deficit (Razmi et al., 2017).

Treatment with SA was able to mitigate the damage caused by WD in the foliar tissue. These changes demonstrated the role of SA in the leaf, contributing to the improvement of physiological processes, maximizing Pn and gs, similar to the result found by Semida et al. (2017).

Water deficiency induces less foliar water loss, through a significant reduction in transpiration rate due to the decrease in stomatal conductance and increase in stomatal resistance due to stomatal closing. In water stress conditions, a higher transpiration rate logically implies a reduction in WUE, which is generally very harmful, specially in extreme drought conditions (Nemeskéri and Helyes, 2019).

Similar results were observed by studies indicating that the application of SA under water stress conditions can keep the stomata open and regulate the hydraulic conductivity (Lobato et al., 2021).

The results recorded with potato plants, also showed a close relationship between SA application and all the photosynthetic parameters assayed. SA application seems to act bringing a protective effect to the plants, increasing its tolerance capacity against WD effects.

Sadeghipour and Aghaei (2012) observed that the decreases in stomatal conductance were lower in plants treated with SA than in control for both common bean cultivars, both in well irrigated and water stress conditions.

Restriction of the decrease in stomatal conductance by the application of SA has an important role in maintaining photosynthetic activity and the damages are reduced (Idrees et al., 2010).

Under restricted water supply conditions, the internal CO₂ level is reduced due to the premature closing of the stomata and the continuous exposure to sunlight causes the transference of electrons to the molecular oxygen, resulting in the generation of superoxide ions in PSI level through the process called Mehler reaction (Ahanger et al., 2017).

In black-eyed peas plants under WD conditions and with salicylic acid application, a large dependency on stomatal closing was evidenced, which reduces the available internal CO₂ and restricted water loss by transpiration. It is effective for the prevention of big losses in foliar water potential (Sadeghipour and Aghaei, 2012).

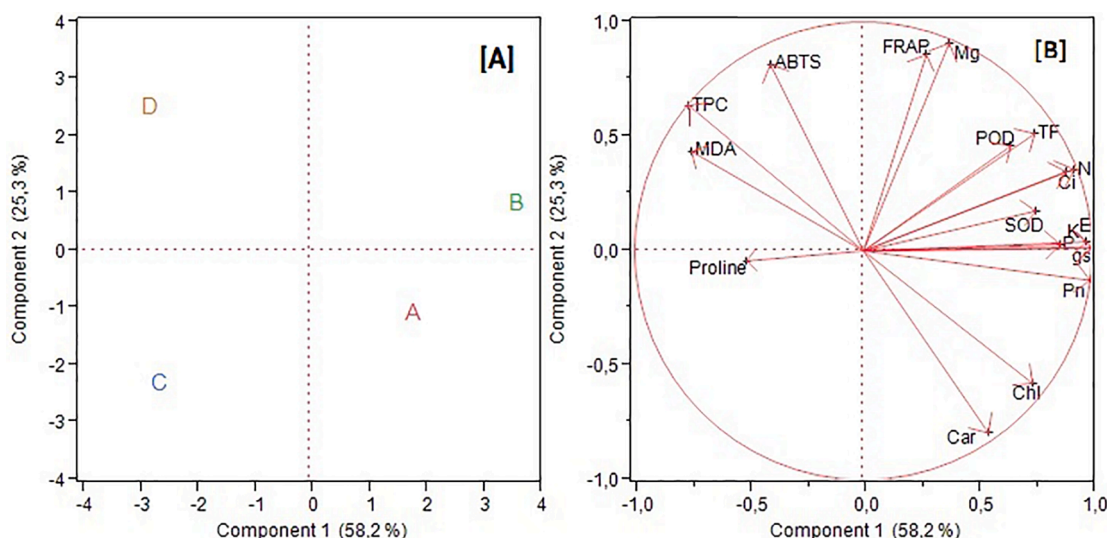


Fig. 10. Score chart [A] and load plot [B] of leaf principal components analysis on 4 combinations of water deficit levels and foliar application of SA in potato at 60 DAE. Sample (Treatment): Control-CK [A], CK+SA [B], WS [C], WS + SA [D]. Abbreviation: total phenolic compounds [TPC], total flavonoids [TF], malondialdehyde [MDA], superoxide dismutase [SOD], peroxidase [POD], proline [Prol], total chlorophyll [Chl], carotenoids [Car].

Table 7

Factor loadings, eigenvalues and proportion of variation associated with three principal components (PC) of the PCA of 10 parameters in 4 treatments in potato tuber.

Characteristics	PC1	PC2	PC3
TPC	0.826	0.479	0.115
TF	0.882	0.188	0.381
MDA	0.557	0.599	0.393
FRAP	0.658	0.486	-0.545
ABTS	0.878	-0.064	-0.259
% starch	0.657	-0.497	0.299
N	-0.018	-0.335	0.919
P	-0.259	0.819	0.273
K	0.761	-0.400	-0.205
Mg	0.399	-0.805	-0.044
Eigenvalue	4.22	2.70	1.73
Percent (%)	42.21	27.07	17.32
Accumulative (%)	42.21	69.27	86.60

Abbreviation: total phenolic compounds [TPC], total flavonoids [TF], malondialdehyde [MDA].

Shah Jahan et al. (2019) observed that foliar application of SA in tomato plants under heat stress conditions presented an increase in the efficient use of water in the leaf of the plant in comparison to control.

To evaluate the nutritional condition of the plants, the main macronutrients (N, P, K and Mg) were analyzed in leaf tissue and tubers of potato plants, maintained or without WD. WD, as expected, caused disturbances in plant nutrition for all evaluated ions. This result is probably directly related to the lesser availability of water in the soil, and considering that the modality of absorption of these elements is directly dependent on the mass flow. The results in plants treated with SA reveal an inversion of this effect with improvement in the levels of elements found in leaves and tubers. According to the Potash and Phosphate Institute (Atlanta, GA), the premature absorption of nitrogen under low humidity condition increases the development of the aerial part and root, which is critical for the final yield. When the soil remains a prolonged period of drought, nitrogen mobility is severely restricted by the dehydrate soil. Thus, when a plant is submitted to a WD, nitrogen deficiency occurs, which rapidly inhibits plant growth and leads to chlorosis (Jonhston, 2002).

Bänziger et al. (2000), arguments that approximately 50% of all N in the leaf is directly involved in the photosynthesis metabolic path or as chlorophyll structure. Thus, if the N supply is insufficient,

photosynthesis rates is reduced, which alters foliar area and consequently accelerate the foliar senescence.

In the study with potato plants, it was possible to correlate disturbances with nitrogen absorption as a function of WD and some photosynthetic parameters, in addition to leaf pigments. Due to the imposition of WD, there was a decrease in photosynthetic rates after 60 days of treatment with deficient water depth, when the application of SA slightly improved the rates; in the same period, the application of SA was not able to positively change the photosynthetic rates as a function of WD. Note that WD caused a decrease in N absorption for the two periods studied (30 and 60 DAE) and for the two water depths (C and WD). However, in all conditions (water and DAE), the application of SA reversed the effect of stress, normalizing the absorption of N. This effect probably favored the metabolism and nutrition of the plants with reflections on the other evaluated parameters. Phosphorus (P) is mineral macronutrient required maintain normal growth rates. It also has an important role in the conservation and transference of energy on the cellular metabolism (Ammann and Blatt, 2009).

A good water supply is necessary for the availability and absorption of phosphate by the plants. Phosphate ions move through the soil mainly by diffusion and if the soil water content decreases, the radius of the water-filled pores are reduced, and the tortuosity increases and P mobility decreases (Faye et al., 2006).

Water deficiency causes a reduction in the absorption and transport of P in the plants. The reduction of available P forms and the increase of P included in the soil reduce P absorption and, consequently, induce a lower foliar P content (Sardans and Peñuelas, 2004). The same authors evidence that a reduction of 22% in soil humidity produced a decrease of 40% in the P content accumulated in the plants, specially because there was a lower increase in aerial biomass. In potato plants, was concluded that this element showed a decrease in absorption rates when comparing the control and WD treatments, probably due to the decrease in water availability. In this case, the application of SA showed a direct and beneficial relationship in plants submitted to WD, improving the absorption of the element. Potassium (K) is a mineral element absorbed in large quantities by the plants and has an important role in regulating the state of the water (Mengel et al., 2001). The potassium ion is involved in several physiological processes, such as enzymatic activation, protein synthesis, photosynthesis, osmoregulation, cellular extension, stomatal movement and other processes (Farooq et al., 2009). The water conditions in plants influence the accumulation of K^+ in laves and interact with the nutritional state of K^+ in some vegetable species

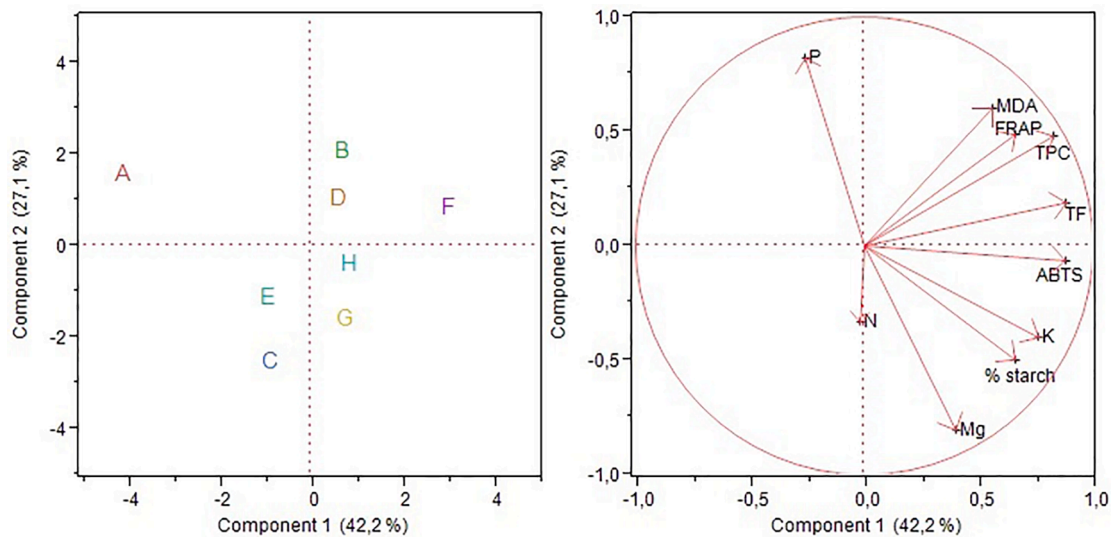


Fig. 11. Score chart [A] and load plot [B] of tuber principal components analysis on 8 combinations of water deficit levels and foliar application of SA in potato at 60 and 90 DAE. Sample (Treatment): CK+SA – 60 DAE [A], CK+SA – 90 [B], CK –60 [C], CK-90 [D], WS –60 [E], WS – 90 [F], WS+SA- 60 [G], WS+SA-90 [H]. Abbreviation: total phenolic compounds [TPC], total flavonoids [TF], malondialdehyde [MDA], phosphorus content [P], potassium content [K], nitrogen content [N], magnesium content [Mg].

(Restrepo-Diaz et al., 2008).

The opening and closing of K^+ channels are of particular importance for the guard-cells and this action mechanism is controlled by the reception of red light, which induces the stomatal opening (de Mello Prado, 2021). Mahouachi (2007) evidenced reduced K^+ levels in banana plants in drought conditions.

Ion magnesium is related in the conservation and conversion of energy, protein synthesis, and act as cofactor in many enzymatic processes. Such processes involves phosphorylation, dephosphorylation, hydrolysis of several compounds and also required to structural stabilizer for several nucleotides (Amtmann and Blatt, 2009; Merhaut, 2006).

The present experiment recorded that the accumulation of magnesium in potato leaves was higher in plants submitted to foliar application of SA.

Brown et al. (2006) found decreases in Mg absorption in both roots and aerial parts of *Spartina alterniflora* (costal grass) maintained under drought conditions. Considering that Mg is less available in WD conditions, the plants roots are not able to absorb the adequate amount of this nutrient to sustain regular plant growth.

MDA concentration and the damage to the cellular membranes increased in WD conditions mainly due to the increase in ROS synthesis (Kundu et al., 2020). MDA contents was detected by the plants grown in WD treatment. With foliar application of SA, the amounts of MDA was reduced.

MDA triggers the action of free radicals and the bioactive compounds have a fundamental role in protecting against them (Moharekar et al., 2003). The authors argue that a MDA increase was detected in potato plants under water deficit conditions, which corroborates previous studies (Razmi et al., 2017). Lower MDA contents was detected in potato leaves, with the exogenous application of SA and under water WD, suggesting it is working to prevent the lipid peroxidation damage in leaf cells.

In general, the secondary metabolites have an important role to protect the plants against environmental stresses, such as high light intensity or UV, mechanical damage, low nutrient availability, cold, pathogen incidence and drought (Akula and Ravishankar, 2011).

In many plants species, the concentration of phenolic compounds increased when the plants were subjected to water stress. For example, the drought induced the production of phenolic compounds in leaves of wheat varieties with resistance to water stress (Hameed et al., 2013).

In maize leaves, Phenylalanine ammonia-lyase (PAL) activity a key

enzyme for the synthesis of phenolic compounds, and total phenolic content were increased in resistant genotypes when the crop was subjected to drought in the five-leaf stage (Hura et al., 2008).

However, the phenolic compounds were reduced under water stress in some crop species, such as cherry tomatoes (Sánchez-Rodríguez et al., 2011).

In different studies, the accumulation of flavonoids is also reported as ROS reducing molecule under water deficit conditions (Nakabayashi et al., 2014; Treutter, 2006).

Increase in antioxidant capacity under water stress is variable, depending on the synthesis activity and distribution of different antioxidant compounds in the plant, and also other factors, for example, the duration and intensity of the stress (Reddy et al., 2004).

Among the phytochemical with antioxidant activity, the phenolic compounds are one of the most important groups and many authors attribute their antioxidant proprieties to their relative abundance in different tissues (Fares et al., 2010).

Results obtained in this research indicate that water deficiency by itself increased the synthesis of bioactive compounds, such as phenolic compounds and flavonoids, in the aerial part of the potato plant. The application of SA generated a moderate control of the secondary metabolites synthesis. This behavior was supported by the evident increase in the capacity to eliminate radicals from the different antioxidant assays.

SA could induce the expression of genes responsible for enzymes involved in the accumulation of antioxidant metabolites, such as terpenoids and polyphenols, including flavonoids (Kumar, 2014).

Under abiotic stress, SOD (superoxide dismutase) is the first antioxidant enzyme that defends the cell against the oxidation that converted the superoxide radicals (O_2^-) in less toxic agents, such as H_2O_2 (Gill and Tuteja, 2010).

Some studies evidenced that SA is involved in the response to water deficiency impact through the improvement of the enzymatic antioxidant defense (Ababaf et al., 2021).

These results discussed in our study, confirm the hypothesis that SA play a important role in regulating enzymatic antioxidant activities to protect the plants from oxidative damage induced by water deficiency (Wani et al., 2017).

Application of SA also promoted increases in SOD, CAT and POD activities in tomato plants under water deficiency, which was explained by the fact that the antioxidant responses induced by SA are essential to

protect the plant from oxidative stress (Fan et al., 2022). Sedaghat et al. (2017) also detected increases in POD activity in wheat plants treated with SA and under water deficiency, confirming that SA can stimulate the antioxidant enzymatic mechanism.

High free proline levels maintain low water potential in plants, promoting the absorption of water from the environment (Alhoshan et al., 2019). Exogenous application of SA stimulated the accumulation of L-proline in potato plants submitted to water deficiency, indicating its functioning for osmoprotection and restoring tissue water.

Similar results were also reported in other plants exposed to the treatment with SA in water deficit conditions (Razmi et al., 2017; Shah Jahan et al., 2019). The results showed that the increase in water content and proline in the tissues after SA supplementation under water deficit stress conditions contributed to the increase in the plants water stress tolerance.

5. Conclusion

The results of the research showed that the right soil water tension and foliar application of salicylic acid effectively alleviated the adverse effects of water deficiency in potato plants, as evidenced by the decrease in MDA accumulation in the tissues, increase in bioactive compounds and antioxidant activity. Thus, the treatment with salicylic acid is beneficial to alleviate abiotic stress like water deficiency. Therefore, the use of the treatment with salicylic acid can be a complementary method to protect plants in water deficit conditions. Furthermore, the research will help us better understand the physiological and biochemical mechanism of tolerance to water deficit stress in potato plants and the related pathways of metabolic regulation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgement

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