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Photosynthesis, biochemical activity, and leaf anatomy of tree tomato (*Solanum betaceum* Cav.) plants under potassium deficiency

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Summary

The effects of potassium (K) deficiency on the physiological, biochemical, and anatomical parameters of leaves in the tree tomato plants (*Solanum betaceum* Cav.) were evaluated during vegetative growth. The experiment was carried out for 135 days after treatment applications under greenhouse conditions, employing the nutrient solutions with the following treatments: control plants (without K deficiency) and the plants with K deficiency. The light response curve, photosynthesis at light saturation (*Amax*), light compensation point (*Ic*), transpiration rate (*E*), stomatal resistance (*SR*), and pigment contents in leaves were evaluated. Additionally, the maximum photochemical efficiency of PSII (*Fv/Fm*), contents of malondialdehyde (MDA), total soluble sugars, proline, and leaf anatomy parameters were assessed.
In the K-deficient plants, the reduction in *Amax* (66%), *Ic* (63.7%), *E* (66%), *Fv/Fm* (17.3%), contents of total chlorophyll (77.4%) and chlorophyll a (52%), thickness of leaf blade *L* (28.5%), palisade parenchyma *PP* (6.5%), and spongy parenchyma *SP* (9.5%) were observed, compared to the control plants. In contrast, the variables that increased significantly were *SR* (65%), MDA (52%), Upper epidermis thickness (*Ue*) (27.1%), and Lower epidermis thickness (*Le*) (22.3%). The potassium deficiency caused alterations in the plant development due to the influence on physiological, biochemical, and anatomical parameters, which suggests the importance of mineral nutrition with K for this plant.

Introduction

The tree tomato (*Solanum betaceum* Cav.), also known as tamarillo, belongs to the Solanaceae family, originating from the Andean forests of southern Bolivia and northern Argentina (ACOSTA-QUEZADA et al., 2015). It is a perennial plant that reaches around 2 to 3 m height in its natural habitat and has a semi-woody stem that ramifies and forms the crown. The tree tomato is one of the most important fruit crops in the Colombian Andean region, with a national production of 196,558 t (ASOHOFRUCOL, 2020), due to its potential for food processing, pigment, cosmetics, and pharmacy industries, fresh consumption, and antioxidant content (ACOSTA-QUEZADA et al., 2015; MOHD NOR et al., 2018).
Chemical and biological factors can affect crop development, including nutrient management (GARZA-ALONSO et al., 2019). Mineral nutrition plays a key role in the growth and development of plants and, consequently, in crop production. Potassium (K) is one of the crucial nutrient elements for meristem functioning, defense, signaling, and transport processes (ARMENGAUD et al., 2009; DEMIDCHIK, 2014). In addition, this element participates in stomatal opening, movement of solutes via phloem, cellulose synthesis, osmoregulation, induced re-

sistance to pathogen attack (DEMIDCHIK, 2014), and water absorption (HAWKESFORD et al., 2012). It participates in about 60 enzymatic reactions, involving photosynthesis, respiration, protein synthesis, and carbohydrate metabolism (DONG et al., 2010). It is also a key element for the establishment of transmembrane pH gradient required for ATP synthesis (HAWKESFORD et al., 2012; ZÖRB et al., 2014).
Various experiments in hydroponics systems and in pots with a substrate lacking K show a negative effect of the K deficiency on the physiological and growth parameters of plants (WANG et al., 2015; SRINIVASARAO et al., 2016; DU et al., 2019). The absence of K altered the distribution of assimilates and this translated into the changes in metabolite contents in vegetative organs (HAWKESFORD et al., 2012). However, in the K-deficient leaves, a concentration of internal CO_2 increased, indicating that the reduction in photosynthesis was more affected by the resistance of leaf mesophyll than by the stomatal resistance (RÖMHELD and KIRKBY, 2010; ZÖRB et al., 2014). In addition, along with a decrease in the K^+ concentration in leaves, not only the photosynthesis rate and RuBP carboxylase activity decreased but also the photorespiration rate (HAWKESFORD et al., 2012). In this way, the diagnostics of the nutrient status of K in plants is important for the optimal production and quality of the crops (MATTIELLO et al., 2015; LU et al., 2016; SANADI et al., 2018). Given its importance as an exotic fruit crop on the international markets (ACOSTA-QUEZADA et al., 2015), the tree tomato has positioned itself as one of the main fruit and vegetable crops produced in Colombia (ASOHOFRUCOL, 2020) due to its high potential for bioprospecting. To our knowledge, there were no published reports on the effects of K deficiencies in the tree tomato at the physiological, biochemical, and anatomical levels.
Due to the above, it would be necessary to characterize the effects of K deficiency on photosynthesis, biochemical composition (malondialdehyde (MDA), proline, total sugars), and leaf anatomy in the tree tomato plants. Therefore, the objective of this study was to evaluate the effect of K deficiency on the physiological, biochemical, and anatomical parameters in the tree tomato plants during vegetative growth.

Material and methods

Plant material and growth conditions
The experiment was carried out under the 7-gauge polyethylene plasticized greenhouse, with daily average air relative humidity of 70%, average temperature of 20.3 °C, and photosynthetically active radiation (PAR) of 200 μmol photons m^2 s^{-1}.
Three-month-old Common Red ecotype tree tomato (*Solanum betaceum* Cav.) seedlings were used, which were subjected to root wash with distilled water to remove soil particles from the substrate. Subsequently, these were transplanted into the black plastic bags with 8 kg capacity (0.40 × 0.60 m) containing quartzite sand of two par-

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ticle sizes (0.7 and 1.5 mm) in a 1:1 v/v ratio, electrical conductivity (EC) of 0.012 dS m^{-1} s^{-1}, and pH of 6.7. Each bag was positioned 1 m apart to avoid the effects between the plants. Once transplanted, these were subjected to pretreatment (acclimatization) for one month, by supplying the complete modified nutrient solution of HOAGLAND et al. (1938), where the control treatment received the complete nutrient solution without mineral deficiencies (Tab. 1).

Tab. 1: Concentration of mineral elements (ml of the stock solutions 1 M to be applied to 7.5 L H_2O) modified based on the stock solutions (HOAGLAND et al., 1938) and the nutrient requirements reported for the species (FISCHER and MIRANDA, 2012).

MACRONUTRIENTS (ml) Treatments Source	Without K	Control
NH_4NO_3	69.72	55.78
KH_2PO_4	0.00	13.02
$Ca(H_2PO_4)_2 \cdot H_2O$	32.16	32.16
KCl	0.00	43.94
KNO_3	0.00	37.86
$CaCl_2$	2.70	2.70
$Ca(NO_3)_2 \cdot 4H_2O$	23.00	23.00
$CaSO_4 \cdot 2H_2O$	20.96	12.58
$Mg(NO_3)_2 \cdot 6H_2O$	41.19	51.48
$MgSO_4$	4.83	0.00
K_2SO_4	0.00	31.83
MICRONUTRIENTS		
H_3BO_3	0.28	0.28
$MnCl_2 \cdot 4H_2O$	0.70	0.70
$ZnSO_4$	0.24	0.24
$CuSO_4 \cdot 5H_2O$	0.19	0.19
$FeSO_4 \cdot 7H_2O$	0.73	0.73

Treatments

The Hoagland solution (HOAGLAND et al., 1938) was modified and adjusted based on the needs of the crop (FISCHER and MIRANDA, 2012) (Tab. 1); this was prepared in distilled water with an EC < 3 μS m^{-1} to generate the stock solutions; later it was diluted in 7.5 L of water to carry out the applications. The following two treatments were used: without K deficiency (control) and with K deficiency (without K), which were applied to the plants two times per week. A volume of 100 ml of the nutrient solution was supplied per plant (according to the substrate moisture retention curve) during vegetative growth.

Physiological parameters
Photosynthesis
At the end of the experiment (135 days after the treatment application (dat)), three plants were taken per treatment and light response curves (*A/PFD*) were obtained with an IRGA LCiPro + kit (BioScientific Ltd. Hoddesdon, UK). The measured radiation points were 1,200, 1000, 800, 700, 600, 500, 400, 300, 200, 100, 50, 40, 30, 20, 10, and 0 μmol photons $m^2 s^{-1}$ between 7:00 - 10:00 h (a range of time previously determined, where a highest rate of photosynthesis was observed). The curves were made using the 4th leaf fully expanded of the middle-third stratum of the plants. The data obtained were adjusted using the Mitscherlich hyperbolic model (ALERIC and KIRKMAN, 2005; MELGAREJO et al., 2010; BARRERA et al., 2012). The parameters derived from the *A/PFD* curves were determined as *Amax* = photosynthesis at saturation by light or maximum photosynthesis (μmol CO_2 m^{-2} s^{-1}), *Ic* = light compensation point (μmol photons m^{-2} s^{-1}).

Gas exchange and chlorophyll a fluorescence
In two fully expanded leaves of the middle-third stratum of four plants per treatment, the maximum photochemical efficiency of PSII was measured (*Fv/Fm*) at 0, 15, 30, 45, 60, 75, 90, 105, 120 and 135 dat with a HandyPEA unmodulated Fluorometer (HansaTech instruments, Norfolk, UK) with a saturating PAR of 3000 μmol photons m^2 s^{-1}. In the same plants and leaves, the transpiration rate (*E*) and stomatal resistance (*SR*) were determined between 7:00 and 10:00 am, using a porometer Li-cor Li-1600 Steady (Lincoln, Nebraska, USA).

Biochemical parameters
These were analyzed at the end of the experiment (135 dat).

Photosynthetic pigment contents
In three individual plants per treatment, the leaves were collected from the middle-third stratum for extraction of the total chlorophyll content (total chl) according to LICHTENTHALER and WELLBURN (1983). The 0.05 g leaf tissue without ribs was used and the extraction was carried out with 80% acetone previously kept at -4 °C. Absorbance at 470, 646 and 633 nm was measured using a BIO-RAD Smart SpecTM 3000 spectrophotometer (BIO-RAD, Philadelphia, USA) expressing the results obtained in fresh weight mg g^{-1} (fw), for the concentration of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Total Chl), and carotenoids.

Malondialdehyde content
The content of malondialdehyde (MDA) was determined with thiobarbituric acid based on that described by WANG et al. (2012). The 0.05 g of fresh leaf tissue was obtained from the middle-third stratum of three individual plants per treatment, then used for maceration and extraction, homogenized with 2 ml of 10% trichloroacetic acid (TCA) solution. To 1 mL of the supernatant, 4 mL of 0.5% TBA solution (prepared in 10% TCA) was added and stirred. Subsequently, it was heated at 95 °C for 30 min and then cooled on ice. It was centrifuged at 5000 rpm for 15 minutes. Absorbance was read with a spectrophotometer (BIO-RAD Smart SpecTM 3000, Philadelphia, USA) at 450, 532 and 600 nm. The blank was 10% TCA. The MDA contents was expressed in μmol mL^{-1} fw.

Total sugars
The soluble sugars were extracted according to DUBOIS et al. (1956) modified by MELGAREJO et al. (2010). The 0.05 g of fresh plant material was weighted from leaves obtained from the middle-third stratum of three plants per treatment. Subsequently, it was macerated with liquid nitrogen, then 5 mL of distilled water were added, and it was stirred at room temperature for 60 min. It was centrifuged at 6,000 rpm for 30 min at 12 °C. The 30 μL of the supernatant were taken per sample, 180 μL of distilled water were added, homogenized, and then 200 μL of 80% phenol were added. Subsequently, 1.0 mL of concentrated sulfuric acid was added, stirring in vortex for 1 min. Finally, absorbance was read at 490 nm with a spectrophotometer (BIO-RAD Smart SpecTM 3000, Philadelphia, USA). The content of total sugars was expressed in μg mg^{-1} fw.

Proline content
The proline content was determined according to BATES (1973), with adjustments (MELGAREJO et al., 2010). The 0.05 g of fresh plant material was obtained from the leaves taken from the middle-third stratum of three individual plants per treatment. Subsequently, 5.0 mL of 3% (w/v) sulfosalicylic acid extracting solution was introduced, stirred for 60 minutes at 10 °C, and centrifuged at 6,000 rpm for 30 minutes at 10 °C in darkness. Next, in dark glass tubes, 1.0 mL of the supernatant was placed and 1.0 mL of freshly prepared ninhy-

drin and 1.0 mL of warm glacial acetic acid were added, which were brought to the boil for 60 min. Then these were left at room temperature and 3mL of toluene was added. Finally, the upper phase was collected and the absorbance was read at 520 nm in a spectrophotometer (BIO-RAD Smart SpecTM 3000, Philadelphia, USA), expressing the content of proline in μg g^{-1} fw. The concentration of proline was determined using the standard curve of L-proline from Sigma®.

Leaf anatomy

At the end of the experiment (135 dat), leaf sections (approximately 30 mm^2) were collected from the middle region of the same leaf used for the gas exchange and *Fv/Fm* measurements. The sections were fixed in a mixture (formalin: 10, ethanol: 5, glacial acetic acid: 85), later they were subjected to different solutions of ethanol and HistoChoice® according to MEGÍAS et al. (2018). The paraffin blocks were cut with a model 820 Spencer rotary microtome (American Optical, Delhi, USA), making transverse cuts in the leaves and covering the midrib with a thickness of 13 μm. The cross sections were arranged and fixed on slides, later they were stained with the double stain of Astra-Blue, basic Fuchsin (KRAUS et al., 1998). Digital images with a resolution of 100X were obtained from the assemblies in Olympus® CX31 microscope (New York Microscope Company, NY), with camera adaptation. Using the Image-ProPlus® program (Media Cybernetics, Rockville, MD), the measurements were made of the leaf thickness (*L*), spongy parenchyma thickness (*PS*), palisade parenchyma thickness (*PP*), Upper epidermis (*Ue*), and Lower Epidermis thickness (*Le*).

Experimental design and statistical analysis

A completely randomized design and two repetitions of the same trial were used in time; in the present study, the data of the second repetition in time are presented. Each trial had four replicates and twenty individual plants per treatment.

The data were statistically analyzed using ANOVA to identify the presence of significant differences between the treatment means due to their effects on the evaluated physiological, biochemical, and anatomical variables. The SAS version 9.2 package was used. Once the significant differences between treatment means were found, the Tukey´s multiple comparison test was used ($p \leq 0.05$).

Results

Physiological parameters
Light response curve (A/PFD)

The response curves to light A/PFD indicate the response that plants present under different light intensities (PÉREZ and MELGAREJO, 2014). The tree tomato plants during vegetative growth under potassium deficiency (without K) had a decrease in *Amax* under the different radiation points (PFD), presenting a final value of 2.3 μmol CO$_2$ m^{-2} s^{-1} and differing from the control plants with 6.8 μmol CO$_2$ m^{-2} s^{-1} at 135 dat (Fig. 1 and Tab. 2). The light compensation point (*Ic*) decreased in the plants deficient in K with a value of 12.17 μmol photons m^{-2} s^{-1} as compared with the control, which obtained a value of 35.1 μmol photon m^{-2} s^{-1} (Fig. 1, Tab. 2).

Transpiration (*E*), stomatal resistance (*SR*) and fluorescence of chlorophyll a (*Fv/Fm*)

The tree tomato plants without K registered a significant reduction ($P \leq 0.05$) in the transpiration rate (*E*) at 90, 120, and 135 dat by 45, 58 and 66%, respectively, compared to the control plants (Fig. 2a). The stomatal resistance (*SR*) presented an inverse behavior to the transpiration, that is, the lower was the transpiration, the higher was the stomatal resistance, as a physiological adaptation of plants to avoid water loss. The *SR* significantly increased in the plants without K
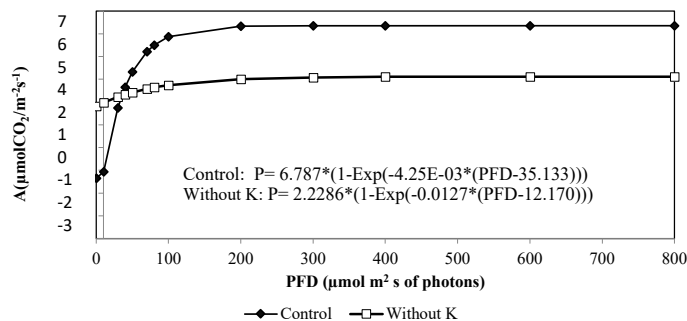
Fig. 1: Light saturation curves of photosynthesis in the tree tomato plants during vegetative growth (A vs. PPFD). Control (without K deficiency) ($R^2 = 0.94$); Plants grown without potassium K (potassium deficiency) ($R^2 = 0.99$). At the bottom of each figure the equation is derived from the fit to a Mitscherlich model. n = 3 plants per treatment.

Tab. 2: Parameters obtained through the light response curve in the tree tomato plants during vegetative growth at 135 dat. Control (without K deficiency) and plants grown without K (potassium deficiency). These were calculated by fitting to a Mitscherlich model. *A max* = photosynthesis at saturation by light or maximum photosynthesis, *Ic* = light compensation point. n = 3 plants per treatment.

Treatment	R^2	*Amax* (μmol CO$_2$ m^{-2}s^{-1})	*Ic* (μmol photons m^{-2}s^{-1})
Control	0.94	6.8±0.044	±35.1
Without K	0.99	2.3±0.047	±12.17

at 120 and 135 dat by 55 and 65%, respectively, compared to the control plants (Fig. 2b). The plants without K registered a significant decrease in *Fv/Fm* at 105, 120 and 135 dat (0.74, 0.72 and 0.67, respectively) unlike the control plants, which remained with an average *Fv/Fm* of 0.81 (Fig. 2c).

Photosynthetic pigment contents

The tree tomato plants grown without K at 135 dat showed a significant reduction in the content of Chl a and total Chl (by 51.7 and 77.4%, respectively) as compared to the control (Tab. 3). However, the plants without K had the tendency to decrease the Chl b content by 33% and a slight increase in the carotenoid content by 14%, unlike the control plants (Tab. 3).

The lipid peroxidation, measured as MDA contents, indicates a possible damage at the cellular membrane level. The plants without K presented significant increases of 0.85 ± 0.07 μmol mL^{-1} MDA, compared to the control, which registered 0.52 ± 0.03 μmol mL^{-1} MDA (Tab. 3).

The contents of total soluble sugars in leaves did not present significant differences between the treatments, however, the tendency to decrease was evidenced in the tree tomato plants cultivated without K (24.87 ± 0.85 μg mg^{-1} leaf fw), unlike of the control plants (29.35 ± 0.98 μg mg^{-1} leaf fw) (Tab. 3).

The content of free proline did not present statistical differences between the treatments; however, an increasing trend was registered for the treatment without K (0.20 ± 0.05 μg g^{-1}), unlike the control (0.16 ± 0.01 μg g^{-1}) (Tab. 3).

Leaf anatomy

The differences were observed in the plants with K deficiency, compared to the control at 135 dat. In contrast to the control, the leaves of the plants grown without K exhibited less thickness of leaf lamina (*L*), which was 28.5% thinner (Tab. 4). The leaves with K deficiency
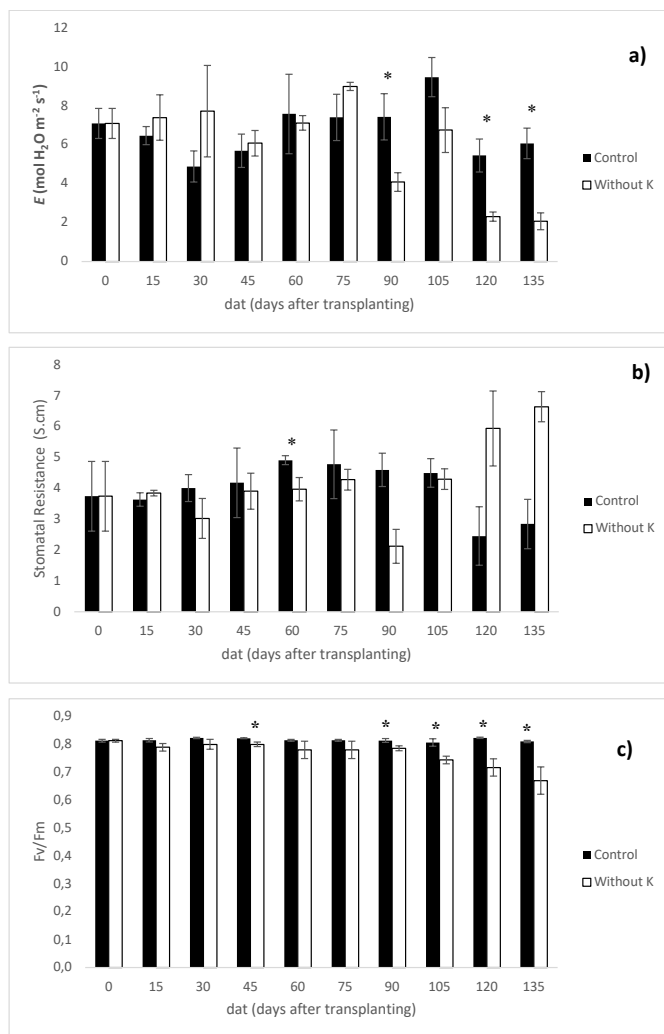
Fig. 2: Physiological parameters in the tree tomato plants during vegetative growth. Treatments: Control (without deficiency) and plants grown without K (potassium deficiency). a) Transpiration rate (E), b) Stomatal resistance (SR); and c) Maximum photochemical efficiency of photosystem II (Fv/Fm). Bars represent the standard error (n = 4 plants per treatment). Significant differences at the 0.05 level of significance are indicated by * (the Tukey's test).

showed a reduction by 6.5% in the thickness of the palisade parenchyma (PP) (Tab. 4) and the cells were irregular (Fig. 4d), unlike in the control (Fig. 4b). The thickness of the spongy parenchyma (SP) was reduced by 9.5%, compared to the control (Tab. 4), and also exhibited an irregular shape of the cells (Fig. 4d). On the contrary, the K deficiency increased the thickness of the Upper epidermis (Ue) and Lower Epidermis (Le), surpassing the control plants by 27.1 and 22.3%, respectively (Tab. 4). The leaves of the K-deprived plants presented variations in the size of the middle vein (Midrib) (data not shown) and had irregular distribution of the vascular bundles (V) and irregular parenchyma cells (P), unlike the control leaves (Fig. 4c, a, respectively).

Discussion

Our results show that the tree tomato plants during vegetative growth under K deficiency severely reduced the photosynthetic rate (Fig. 1 and Tab. 2), presenting a behavior similar to that reported for *Arabidopsis* plants (ARMENGAUD et al., 2009), soybean (DONG et al., 2010) and corn (DU et al., 2019) under deficiency of this element. LU et al. (2016) pointed out that the K deficit affects the photosynthetic rate due to the lower activity of RuBisCO and the activation of the pyruvate kinase, which catalyzes the phosphoenolpyruvate to produce ATP and pyruvate in glycolysis (WANG and WU, 2010). In the same way, the activity of RuBP carboxylase could be negatively affected due to the fact that K maintains a high pH in the stroma and its deficiency alters the photorespiration (HAWKESFORD et al., 2012).

In this study, as the K deficiency progressed, the transpiration rate (E) decreased and stomatal resistance (SR) increased, which indicates that starting from 90 dat the plants experienced a stress, which was reflected with the reduction of Fv/Fm (Fig. 2) and was further translated into a reduction in photosynthesis (Tab. 2). Similar results were reported by TANG et al. (2015) in tea, WANG et al. (2015) in soybean, and DU et al. (2019) in corn, where K deficiency significantly reduced the variable E, unlike in the control plants. This response is due to the important role of K in maintaining turgor pressure, a fundamental variable for stomatal opening (HAWKESFORD et al., 2012). It was, possibly, due to the fact that the reductions in E (Fig. 2a) and $Amax$ (Tab. 2) are caused by the stomatal limitations (RÖMHELD and KIRKBY, 2010; JIN et al., 2011). ANDRÉS et al. (2014) pointed out that the K deficiencies affect the opening of stomata, decreasing the photosynthetic rate due to the limitation in the assimilation of CO_2 from the atmosphere to the internal spaces of leaves, causing a negative effect on the carboxylation sites within the chloroplasts (FLEXAS

Tab. 3: Contents of photosynthetic pigments (mg g^{-1} leaf fw), malondialdehyde (MDA) (µmol mL^{-1}), total sugars (µg mg^{-1} leaf fw), and proline (µg g^{-1}) in the leaves of tree tomato plants during vegetative growth. Means ± standard error (n = 3 per treatment) are presented. Control (without deficiencies) and plants grown without K (potassium deficiency). Different letters indicate significant statistical differences with a P value <0.05 (the Tukey's test).

Biochemical parameter	Chl a	Chl b	Total Chl	Carotenoids	MDA	Total sugars	Proline
Control	0.58 ±0.029 A	0.31 ±0.004 A	0.89 ±0.025 A	0.56±0.060 A	0.52±0.03 A	29.35±0.98 A	0.16±0.01 A
Without K	0.30±0.064 B	0.24±0.042 A	0.54±0.100 B	0.64±0.195 A	0.85±0.07 B	24.87±0.85 A	0.20±0.05 A

Tab. 4: Anatomical parameters registered in the cross sections of the tree tomato leaves during vegetative growth. Control (without deficiencies) and plants grown without K (potassium deficiency). Means ± standard error (n = 3 per treatment) are presented. Different letters indicate significant statistical differences with a P value <0.05 (the Tukey's test).
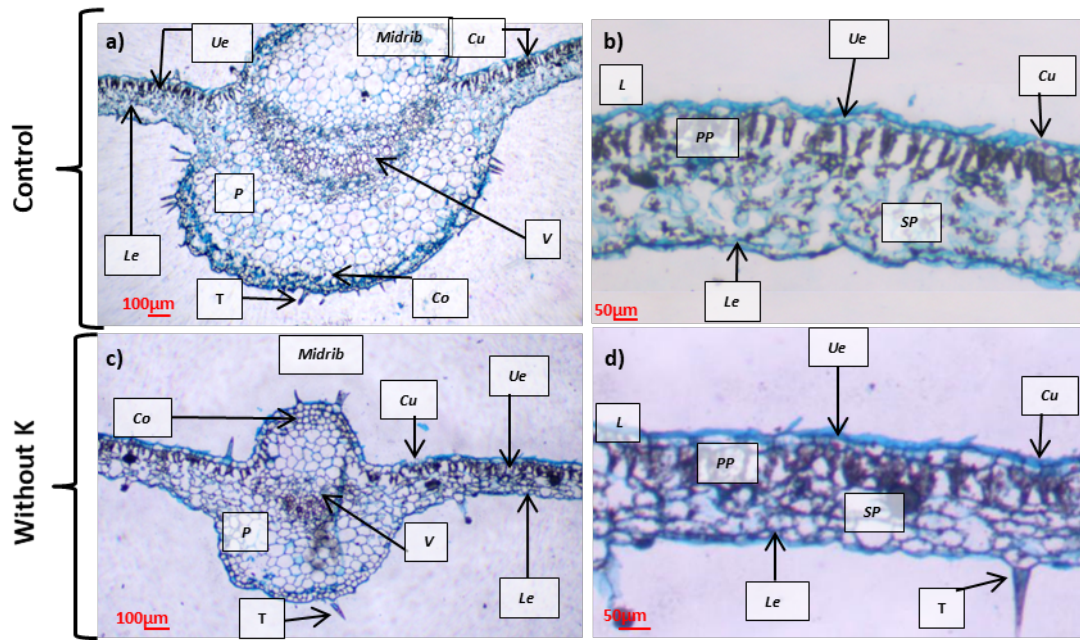
Treatment	Leaf blade thickness (L)	Palisade parenchyma thickness (PP) (µm)	Spongy parenchyma thickness (SP) (µm)	Upper epidermis thickness (Ue) (µm)	Lower epidermis thickness (Le) (µm)
Control	247.6±13.8 B	51.9±2.5 A	87.6±7.8 A	9.6±0.7 B	10.3±0.4 B
Without K	177.1±10.7 A	48.5±2.83 A	79.3±6.1 A	12.2± 0.5 A	12.6±1.21 A

Fig. 4: Cross-sections of leaves in the tree tomato plants at 135 dat. Control plants (without deficiencies) (a, b) and plants grown without K (K deficiency) (c, d). Midrib (a, c), same magnification, Bar = 100 µm. Leaf blade (b and d), same magnification, Bar = 50 µm. *P*: parenchyma; *V*: vascular bundles; *Co*: Colenquima; Midrib: rib; *PP*: palisade parenchyma; SP: spongy parenchyma, *Ue*: Upper epidermis, *Le*: Lower Epidermis, T: trichomes, and Cu: cuticle.

et al., 2008). Similarly, as K is important for the opening of stomata, the lack of this element would reduce its accumulation in the vacuole, affecting the stomatal opening (JORDAN-MEILLE and PELLERIN, 2008) and altering the stomatal and non-stomatal regulation of CO_2 (TANG et al., 2015).

The response of *E* was opposite to *SR* (Fig. 2a, b) as a strategy of the plants to avoid the loss of water inside the leaf towards the atmosphere (QUEZADA et al., 2002). The K deficiency generated a reduction in *Fv/Fm* (Fig. 2c), suggesting a possible photoinhibition starting from day 105 (MAXWELL and JOHNSON, 2000). This photoinhibition was, probably, related to the chlorophyll content (Tab. 3), which agrees with that reported by HU et al. (2016). KITAJIMA and BUTLER (1975) proposed that *Fv/Fm* alterations generate changes in the photochemical conversion of energy on the reaction centers of PSII and induce a possible photoinhibition.

According to our results, it can be inferred that the reduction in the content of pigments, such as Chla, Chlb and total Chl (Tab. 3), due to the treatment without K were related to the low photosynthetic rate (*Amax*) (Tab. 2) and possible stomatal limitations. Results similar to those found in this study have been reported by JIN et al. (2011) and CAVALCANTE et al. (2015), indicating that the K deficiencies could be associated with the degradation of chlorophylls, biochemical alteration of chloroplasts and, therefore, less absorption of light. JIN et al. (2011) and WANG et al. (2012) suggested that the K deficiency generates production of reactive oxygen species (ROS) due to the null or low consumption of ATP and NADPH in the Calvin cycle due to the absence of the electron acceptor $NADP^+$, causing the degradation of the chloroplast pigments (CAVALCANTE et al., 2015). Additionally, the reduction in the chlorophyll contents was, possibly, due to the fact that the K deficiency is associated with the nitrogen deficiencies, which is key element in the synthesis of proteins destined for growth and development. Potassium is involved in the protein synthesis in roots as well as in the root uptake and assimilation of nitrogen (COSKUN et al., 2017). In particular, K participates in the regulation of NRT2 nitrate transporters in roots and the activity of nitrate reductase (COSKUN et al., 2017; HU et al., 2016; ARMENGAUD et al., 2009).

Tab. 3 shows the increase in the MDA content in leaves with the K deficiency. These results agree with that reported by HU et al. (2016) in cotton and DU et al. (2019) in corn, which, under the K deficiency, presented the high MDA contents and were associated with high H_2O_2 production (a variable not measured in the present study). Similarly, HERNANDEZ et al. (2012) found significant differences in tomato with low K deficiency, presenting high accumulation of ROS, simultaneously increasing the amount of MDA, which suggests that K deficiency caused the oxidative damage to lipids and, finally, caused a chlorosis in the plants.

Our results show that the K deficiency decreased the leaf thickness including the thickness of the spongy and palisade parenchyma (Tab. 4) resulting in a decrease in *Amax* (Tab. 2), which shows a close relationship between the leaf anatomy and the photosynthetic process. The diffusion of CO_2 during photosynthesis can be regulated by carbon allocations, which is determined by the leaf thickness and the size of the mesophyll cells (HU et al., 2020). This is in accordance with data reported in corn by DU et al. (2019) who observed that the K deficiency negatively affected the anatomical structure of leaves, significantly reducing the size of the cells. In the same way, LIN and YEH (2008) indicated that the thickness and size of leaf cells are reduced in plants because the water storage tissues in cells decreases along with the increment of K deficiency. The damage to the leaf anatomy, apparently, influences the photosynthetic process, the transport of nutrients and water (MATTIELLO et al., 2015). Faced with the stress due to the K deficiency, the leaves tend to be smaller, and the growth and cell expansion decrease (ARMENGAUD et al., 2009; ELISE et al., 2020). It has been reported that the mesophyll cells reduce the rate of cell division due to the K deficiency (TOSHIO et al., 2009; HU et al., 2020) and K regulates cell expansion and proliferation of the mesophyll, which affects the leaf area (BATTIE-LACLAU et al., 2014; LU et al., 2020). For this reason, the less thickness was observed in the spongy and palisade parenchyma of the tomato tree plants grown without K (Tab. 4). In general, it was observed that the K deficiency negatively affected the physiology, biochemical components, and anatomical parameters in the tree tomato plants.

Conclusions

The tree tomato plants grown with the K deficiency reduced the physiological parameters $Amax$, Ic, E, Fv/Fm, content of chlorophylls a, b and total. On the other hand, these plants increased the SR, and the MDA contents in leaves. Compared with the control plants, the deficiency of K altered the leaf anatomy, reducing the thickness of the leaf along with the thickness of spongy and palisade parenchyma. In addition, an increase in the thickness of the adaxial and abaxial epidermis was evidenced. This research presents the physiological, biochemical and anatomical indicators of the tree tomato plants subjected to potassium deficiency during vegetative growth, which can be used for monitoring and adjusting fertilization programs of this species and, thus, be useful for the tree tomato growers.

Conflicts of interest

No potential conflict of interest was reported by the authors.

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