



Water relations of cassava cultivated under water-deficit levels

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Abstract

The tolerance of plants to water deficit involves a series of adaptive mechanisms; however, little is known about the physiological characteristics of cassava (*Manihot esculenta* Crantz), which is one of the most tolerant crops to adverse environmental conditions. The objective of this work was to evaluate the water relations in cassava plants subjected to different levels of water deficit. The treatments were conducted in three evaluation periods (0, 45 and 90 days after water deficit) and at three soil water tensions (– 10, – 40 and – 70 kPa), with five replicates. The plants were mainly affected at 45 days after the water deficit, with an increase of 42.9% in total chlorophyll content and 35.3% in carotenoid content in plants under a tension of – 70 kPa; however, these plants reduced by 30.8% chlorophyll *a* content at 90 days of the treatments. The water potential, relative water content and electrolyte leakage in the leaf were not altered by the soil water tension. There was an increase of 35.4% in stomatal density independent of soil water status at 90 days and of 16.0% under tensions of – 40 and – 70 kPa; however, the effective quantum efficiency of photosystem II and rate of electron transport were reduced. Cassava can maintain a leaf water potential close to – 0.3 MPa in the predawn and the integrity of the cell membranes in leaves under a soil water tension of up to – 70 kPa.

Keywords *Manihot esculenta* · Drought · Chlorophyll · Water potential · Fluorescence · Stomatal density

Introduction

Cassava (*Manihot esculenta* Crantz) is a perennial plant, a heliophyte, and a tuberous root shrub that belongs to the Euphorbiaceae family and is one of the main food sources of energy in developing countries (Alves 2002). Its starchy tuberous roots are used in human food and feed for animals and as a raw material for the food, textile and paper industries (Santisopasri et al. 2001).

Cassava is a drought-tolerant plant and is commonly produced in areas that receive less than 700 mm of rain per year and have a dry season of 4–6 months (El-Sharkawy 2012). However, the period of greatest susceptibility of the crop to water deficit is approximately 30–150 days after planting (Oliveira et al. 1982). The reduction in yield of tuberous roots depends on the duration of the water deficit and is

determined by the sensitivity of a particular phenological stage to stress (Lebot 2009).

A water deficit can be defined as the entire water content of a tissue or cell that is below the water content of maximum hydration (Taiz and Zeiger 2013). Reduction of water potential affects all activities that depend on turgor, such as cell division and stretching, photosynthesis, respiration, translocation, ion absorption and nutrient metabolism. Photosynthesis is one of the variables that show great sensitivity to drought (Lawlor and Tezara 2009; Inman-Bamber and Smith 2005).

Photosynthetic pigments are often used to estimate the photosynthetic capacity of plants because their contents can vary between species as well as between genotypes of the same species (Baret et al. 2007; Ciganda et al. 2008). In stress environments such as drought, the occurrence of inhibition or reduction in electron transfer at the photochemical stage of plant photosynthesis is reflected by an increase in chlorophyll fluorescence *a* (Maxwell and Johnson 2000), and the excess energy absorbed by the pigments is mainly dissipated because of water deficiency and the limitation in the assimilation of CO₂.

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The reduction of leaf stomatal conductance in plants in a water deficit condition is of paramount importance to avoid severe water loss and thus to protect the photosynthetic systems and cellular structures from irreversible damage (Duque and Setter 2013). The physiological and developmental mechanisms that allow a species to tolerate prolonged periods of water deficit may involve various attributes (Alves and Setter 2004) that depend on the genotype, the stage of plant development, and the duration and severity of the stress (Bray 1993; Santos and Carlesso 1998).

Therefore, the objective of this research was to evaluate the water relations in cassava plants under three soil water tensions during the phenological stage between 90 and 180 days after planting.

Materials and methods

Description of the experimental area and treatments

The experiment was carried out in a protected environment at the School of Agricultural Sciences—FCA/UNESP, located in Botucatu (22°51'S, 48°26'W and 786 m above sea level) in the State of São Paulo, Brazil. The protected environment was a wooden structure and galvanized steel arches with the right foot of 3 m in a total area of 120 m² with anti-aphid fabric of 2 mm diameter on the sides and a transparent plastic of 150 µm in the roof. The climate of the region has been characterized by Thornthwaite's methodology as B_{2r}B₃'a', a humid mesothermic climate with low water deficits (April, July and August) and a concentration of summer potential evapotranspiration of 33%. The annual average air temperature is 20.3 °C, and the annual average rainfall is 1428.4 mm (Cunha and Martins 2009). The soil of the area has been classified as medium texture Neosol (Embrapa 2006).

The cultivar IAC 576-70 was used because it is the main cultivar of sweet cassava cultivated in the State of São Paulo; it occupies approximately 90% of the area, with high yields, excellent culinary qualities and architecture favourable to cultural practices (Otsubo and Lorenzi 2004).

The experiment was conducted in a completely randomized design that consisted of three evaluation periods [0, 45 and 90 days after water deficit (DAD)] and three soil water tensions (− 10, − 40 and − 70 kPa), with five replicates. The evaluation periods occurred during the development phase of the cassava branches and leaves, that is, the period from 90 to 180 days after planting (DAP) (Alves 2006), with 0, 45 and 90 DAD corresponding to 90, 135 and 180 DAP, respectively. A soil water tension of − 10 kPa was considered as the control, a tension of − 40 kPa as a

moderate water deficit and a tension of − 70 kPa as a severe water deficit.

The planting occurred on November 13, 2014 using branches removed from the middle third of healthy plants aged 12 months. The branches were sectioned at right angles with the aid of a machete, which produced a seedbed of 0.20 m in length with 5–7 nodes. A seedbed of 1.00 × 1.00 × 0.57 m was planted per pot that contained approximately 0.30 m³ of soil and allowed the drainage of water.

Puncture tensiometers were installed close to the plant in all the pots at 20 cm of soil depth, and the tension was measured by a digital tensimeter (SondaTerra, São Paulo, Brazil). The plants were inspected and irrigated daily, and soil moisture was maintained at field capacity until the start of the treatments.

The chemical analysis of the soil was run at 0–20 cm depth before the experiment was set up. However, there was no need for fertilization at planting; cover fertilization was performed at 40 DAP with urea at a dose of 40 kg ha^{−1} of N (Lorenzi et al. 1997). Thinning of the stems of the plants was performed at 45 DAP, which resulted in a standard of two stems per pot.

Application of treatments and crop management

The cassava plants were submitted to soil water tensions of − 10, − 40 and − 70 kPa from 90 DAP up to 180 DAP. The readings in the tensimeter were taken between 4 PM and 5 PM and were converted into soil water potential (Ψ_m) in units of kPa (Dourado Neto et al. 1995); the data were adjusted according to the soil water retention curve (Fig. 1) using the model described by Van Genuchten (1980).

The estimation of the irrigation layer was performed based on the available water capacity (AWC) and aimed to

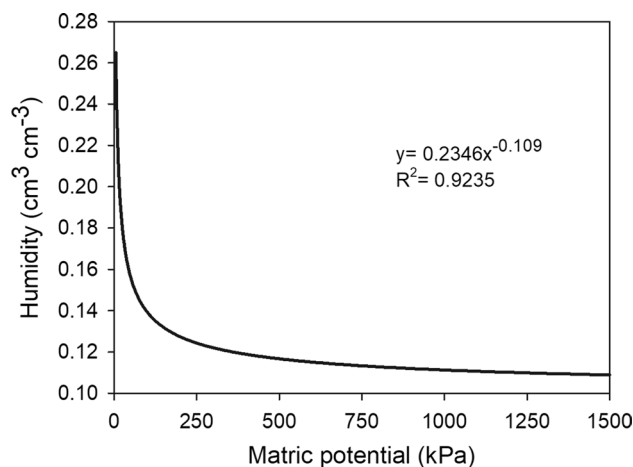


Fig. 1 Soil water retention curve of the experimental area for depths from 0 to 20 cm

maintain the soil water tension in each treatment according to Eq. (1):

$$AWC = (FC - PWP) \times R \quad (1)$$

where AWC is the available water capacity in the soil (mm), FC is the volumetric water content in the field capacity ($\text{cm}^3 \text{cm}^{-3}$), PWP is the volumetric water content at the permanent wilting point ($\text{cm}^3 \text{cm}^{-3}$) and R is the effective depth of the root system (mm) (considered 200 mm).

The meteorological data within the protected environment were recorded in the experimental period by a data logger (Instrutherm, HT-500, São Paulo, Brazil) that obtained the temperature ($^{\circ}\text{C}$) and the relative humidity (%) of the air. The vapour pressure deficit (VPD) of the air was then calculated (Smith et al. 1991).

Physiological variables

The photosynthetic pigments (chlorophyll *a*, *b* and carotenoids), stomatal density, water potential, relative water content, electrolyte leakage and chlorophyll *a* fluorescence were evaluated on leaves at 0, 45 and 90 DAD.

Photosynthetic pigments

The photosynthetic pigments were quantified in fresh leaf tissues by extraction in a solution of *N, N*-dimethylformamide (DMF) and subsequent determination by spectrophotometry. Leaf discs of 1.1 cm diameter were removed from the leaves located in the middle third of the cassava plants and placed in test tubes wrapped in aluminium foil with a screw cap containing 2 mL of DMF. The pigment contents were determined after 24 h, and 1 mL of the extract was then taken and diluted in 1 mL of deionized water for spectrophotometry (Shimadzu, UV-2700, Kyoto, Japan) at wavelengths of 664 nm for chlorophyll *a*, 647 nm for chlorophyll *b* and 480 nm for carotenoids (Porra et al. 1989; Wellburn 1994).

Stomatal density

The stomatal density was determined in the same leaves that were removed to analyse the photosynthetic pigments by impression of the leaves using colourless nail polish and transparent adhesive tape. The number of stomata was obtained by the average count from two impressions of the abaxial surface of the leaf; an area of 0.0744 mm^2 with $40\times$ magnification was considered in a biological microscope (Biovideo, BEL Photonics, Monza, Italy). The stomatal density was estimated by the number of stomata mm^{-2} (Majumdar et al. 1972).

Water potential and relative water content

The leaf water potential (Ψ_w) was determined in the second expanded leaf removed from the apex of the cassava plants between 5 and 7 AM (predawn) using a Scholander pressure chamber (SoilMoisture Equipment, Santa Barbara, CA, USA). The readings were determined when exudation occurred from the cutting performed on the leaf petiole.

The leaf relative water content was obtained by determining on a precision analytical scale the masses of fresh, turgid and dry matter of six leaf discs of 1.1 cm diameter removed from the middle third of the cassava plants. The values of the masses were input to Eq. (2) (Barrs and Weatherley 1962):

$$RWC = (M_f - M_s)/(M_t - M_s) \times 100 \quad (2)$$

where RWC is the relative water content (%), M_f is the fresh matter mass of the leaf discs (g), M_s is the dry matter mass of the leaf discs (g) and M_t is the mass of the turgid matter of the leaf discs (g).

Electrolyte leakage

The integrity of the cell membranes was determined by removing 30 leaf discs of 0.5 cm diameter from the middle third of the cassava plants, which were kept under agitation for 24 h in 10 mL of 0.3 M solution of mannitol. At the end of the incubation, the electrical conductivity of the solution was measured by a digital conductivity meter. The tubes containing the solution and the leaf discs were then weighed and heated to boiling for 10 min. After cooling, the tubes were again weighed, the difference of the evaporated volume was replaced with deionized water and the tubes were agitated for another 30 min. The electrical conductivity was read again after the 30-min period and the electrolyte leakage was calculated by Eq. (3) (Lafuente et al. 1991):

$$EL = (C_i/C_f) \times 100 \quad (3)$$

where EL is the electrolyte leakage (%), C_i is the initial electrical conductivity ($\mu\text{S cm}^{-1}$) and C_f is the final electrical conductivity ($\mu\text{S cm}^{-1}$).

Chlorophyll *a* fluorescence

The emission of chlorophyll *a* fluorescence was evaluated using a portable light modulated fluorometer (Opti-Sciences, OS5p, Hudson, USA) from the initial fluorescence (F_0), maximum fluorescence (F_m) and variable fluorescence (F_v). The evaluations were performed at 9 AM (the time the plants were adapted to the environment with photosynthetically active radiation, PAR, approximately $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$). The effective quantum efficiency of photosystem II ($\Delta F/F_m'$), the electron transport rate (ETR) and the potential

quantum efficiency of photosystem II (F_v/F_m) were analysed, the last being obtained after the leaves were allowed to adapt to the dark for ~ 20 min with the use of leaf clips and just after a saturating light flash of 0.5 s duration. All readings were standardized on the third expanded leaf of the plant from the apex, and an average of two readings in each plant was obtained.

Statistical analysis

Data were submitted to analysis of variance by the F test with subsequent comparison of means using the Tukey test ($p \leq 0.05$) using the statistics program SISVAR (Variance Analysis System, version 5.0, Lavras, Brazil) (Ferreira 2008). Data were analyzed in a split plot in a time arrangement, considering the plot as the evaluation period and the split plot as the soil water tension. When there was no interaction between the factors under study, the data were evaluated separately.

Results

Environmental conditions during the experiment

The air temperature within the protected crop during the experiment ranged from 21.0 to 33.3 °C, with an average of 23.5 °C in the period from 90 to 180 DAP. The relative humidity of the air averaged 67.2%; however, the humidity was higher (73.9%) in the development phase of the branches and leaves of the cassava (90–180 DAP) (Fig. 2a). The increase of the relative humidity of the air in this phase and the consequent decrease in temperature directly contributed to the reduced VPD of the air, which ranged from 0.6 to 1.0 kPa. The VPD reached an average of 2.4 kPa at 45 DAP (Fig. 2b).

From 90 to 180 DAP, the IAC 576-70 sweet cassava plants were irrigated daily to maintain the soil water tension in each treatment. The tensions before irrigation during this period were – 17.6 kPa in the treatment of – 10, – 48.9 kPa in the treatment of – 40 and – 65.3 kPa in the treatment of – 70 kPa. The soils took approximately 15 days after the start of the treatments to reach the soil water tensions of – 40 and – 70 kPa, which were maintained until the end of the experiment (Fig. 2c).

Physiological analyses

At the start of the treatments, when all cassava plants were under a soil water tension of approximately – 10 kPa, the chlorophyll *a* contents were on average 11.18 $\mu\text{g cm}^{-2}$ (Fig. 3a). There was no significant difference between the plants under – 10 and – 40 kPa at 45 DAD; however, plants

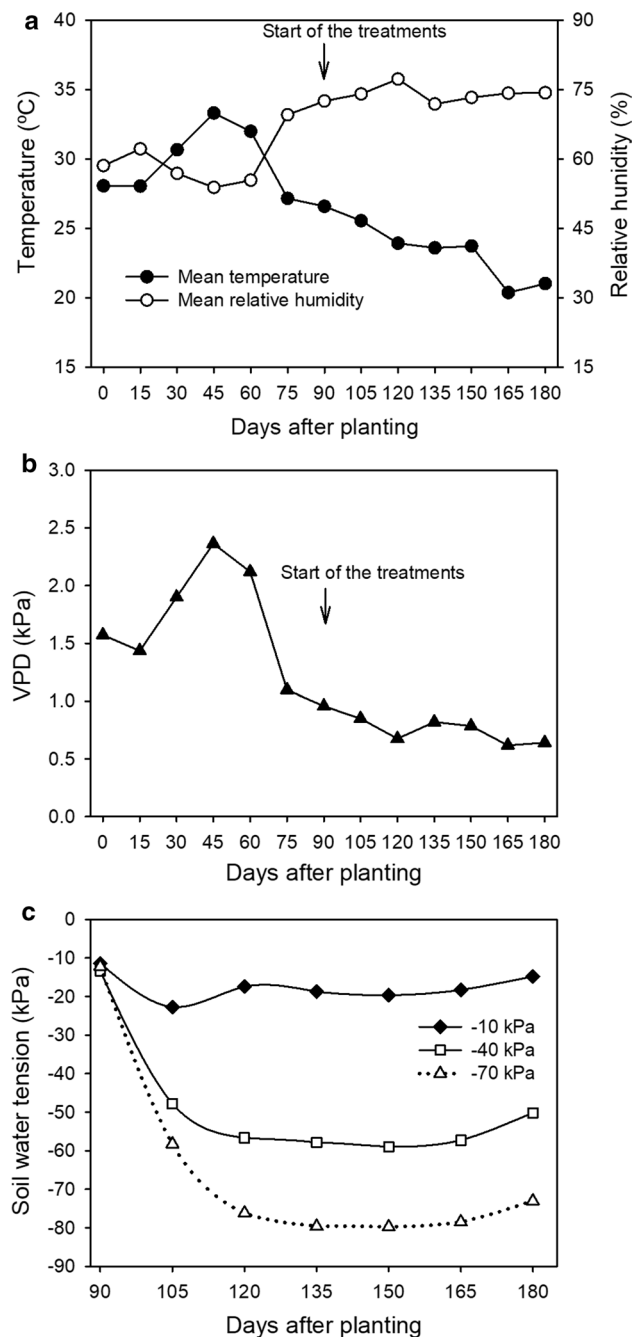


Fig. 2 Biweekly temperature and relative humidity (a), vapour pressure deficit (b) and soil water tension in the experimental period (c) during the development phase of the branches and leaves of the sweet cassava IAC 576-70

under – 70 kPa showed 18.87 $\mu\text{g cm}^{-2}$ of chlorophyll *a*, an increase of 50.4% in relation to plants under a tension of – 10 kPa in the same period. There was no significant difference in the chlorophyll *a* levels of cassava plants independent of soil water tension at 90 DAD. In general, plants under – 10 kPa maintained average chlorophyll *a* levels of 11.88 $\mu\text{g cm}^{-2}$ during the development phase of the

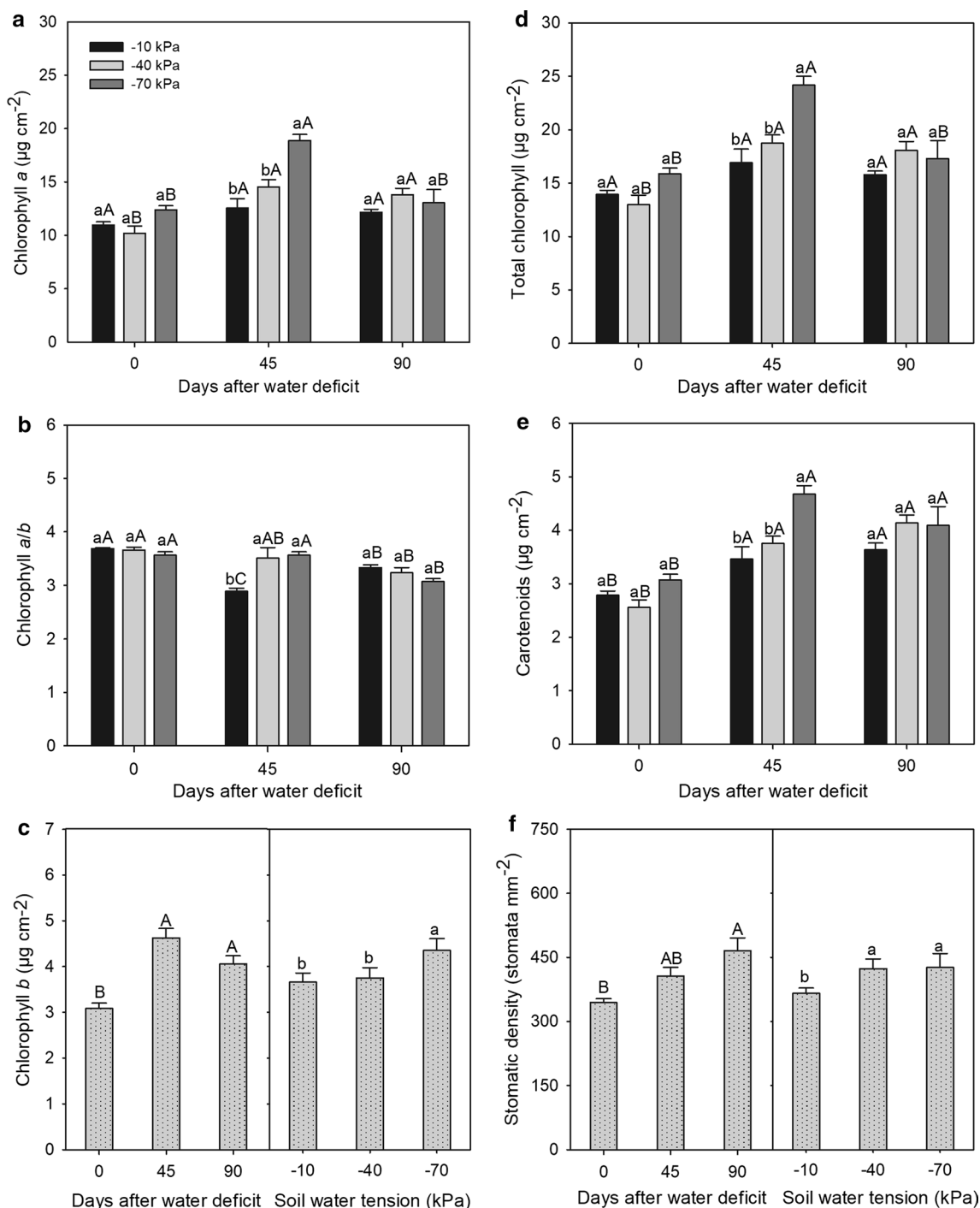


Fig. 3 Chlorophyll *a* (a), chlorophyll *a/b* (b), chlorophyll *b* (c), total chlorophyll (*a + b*) (d), carotenoids (e) and stomatal density of the foliar abaxial surface (f) in IAC 576-70 sweet cassava plants under different soil water tensions during the development phase of the

branches and leaves. Means followed by the same capital letter for the days and lowercase letter for the tensions did not differ based on the Tukey test ($p \leq 0.05$). The bars indicate the standard error of the mean of five replicates

branches and leaves, whereas the plants under tension of -40 kPa had an increase of 42.5% at 45 DAD and an average of $14.17 \mu\text{g cm}^{-2}$ chlorophyll *a* between 45 and 90 DAD. Plants under a tension of -70 kPa increased 52.3% at 45

DAD relative to the plants at the start of the treatments, with a reduction of 30.8% in chlorophyll *a* content at 90 DAD.

The chlorophyll *a/b* ratio of cassava plants averaged 3.64 at 0 DAD (Fig. 3b). Plants under a tension of -10 kPa had a

lower chlorophyll *a/b* (2.88) ratio at 45 DAD, which differed from other plants under tensions of -40 and -70 kPa and indicates that they had higher levels of chlorophyll *b* in their leaves. There was no significant difference between plants at different soil water tensions at 90 DAD. The chlorophyll *a/b* ratios of plants under a tension of -10 kPa fluctuated during the experimental period, with significant differences between the days after the water deficit. Plants under tensions of -40 and -70 kPa had a decrease in chlorophyll *a/b* ratio between 0 and 90 DAD.

The levels of chlorophyll *b* in the leaves showed no interaction between the DAD and soil water tension (TS) factors ($F = 1.75$; $p = 0.17$). The contents increased during the development phase of the branches and leaves of the cassava plants regardless of the soil water tension applied, with a mean of $4.62 \mu\text{g cm}^{-2}$ chlorophyll *b* at 45 DAD and $4.06 \mu\text{g cm}^{-2}$ at 90 DAD. In general, the plants under a tension of -70 kPa presented a chlorophyll *b* increment of 18.8% in relation to the plants under a soil water tension of -10 kPa (Fig. 3c).

The total chlorophyll content did not significantly differ among the cassava plants at 0 DAD; the average was $14.27 \mu\text{g cm}^{-2}$ (Fig. 3d). At 45 DAD, the plants submitted to a tension of -70 kPa had total chlorophyll contents of $24.17 \mu\text{g cm}^{-2}$, an increase of 42.9 and 29.1% in relation to plants under tensions of -10 and -40 kPa, respectively, with significant differences between the tensions. The plants had the same total chlorophyll content, a mean of $17.05 \mu\text{g cm}^{-2}$, at 90 DAD regardless of the soil water condition. Plants under a tension of -10 kPa maintained total chlorophyll contents of approximately $15.55 \mu\text{g cm}^{-2}$ during the development phase of the branches and leaves, whereas plants under a tension of -40 kPa showed an increase of 44.1% at 45 DAD in relation to the beginning of the treatments and did not differ from the plants in the same tension at 90 DAD. Plants under a tension of -70 kPa had a decrease of 28.4% in total chlorophyll content at 90 DAD in relation to 45 DAD.

The carotenoid content of cassava plants at 0 DAD was on average $2.80 \mu\text{g cm}^{-2}$ (Fig. 3e). The plants under a tension of -70 kPa had average carotenoid levels of approximately $4.67 \mu\text{g cm}^{-2}$ at 45 DAD, 35.3% higher than the plants under a tension of -10 kPa, which significantly differed from this case and the plants under a tension of -40 kPa. There was no significant difference of the plants in the different tensions at 90 DAD. All plants presented increase of carotenoid contents between 0 and 90 DAD; at the end of the experimental period, $3.63 \mu\text{g cm}^{-2}$ was obtained in the plants under a tension of -10 kPa, $4.13 \mu\text{g cm}^{-2}$ in the plants under a tension of -40 kPa and $4.08 \mu\text{g cm}^{-2}$ in plants under a tension of -70 kPa.

The stomatal density of the abaxial surface of the cassava leaf varied from 344.08 to 465.95 stomata mm^{-2}

during the development phase of the branches and leaves regardless of the soil water condition. However, the water deficiency caused by soil water tensions of -40 and -70 kPa stimulated a 16.0% increase in leaf stomatal density (Fig. 3f). There was no interaction between the DAD and TS factors in the stomatal density variable ($F = 2.07$; $p = 0.11$).

The impression of the abaxial surface of the cassava leaf verified at 90 days of treatment under a soil water tension of -10 kPa demonstrated turgid and more spaced stomata, which evidenced the occurrence of a crown of papilliform cells around the stomata (Fig. 4a). The plants under a tension of -40 kPa presented partially open stomata and greater counts (Fig. 4b). The tension of -70 kPa stimulated the closure of the stomata in the leaves and the production of smaller stomata with less spacing between them (Fig. 4c).

The interaction between the DAD and TS factors did not occur in the physiological variables of water potential ($F = 1.28$; $p = 0.30$), relative water content ($F = 1.26$; $p = 0.31$), electrolyte leakage ($F = 1.83$; $p = 0.15$), potential quantum efficiency of photosystem II ($F = 0.01$; $p = 0.99$), effective quantum efficiency of photosystem II ($F = 1.04$; $p = 0.40$) and electron transport rate ($F = 2.34$; $p = 0.08$).

The water potential (Fig. 5a) and relative water content (Fig. 5b) in the leaves diminished to 90 DAD independent of soil water status and showed a decrease of 48.2% in water potential and 8.5% in relative water content between 45 and 90 DAD. The different soil water tensions did not interfere in the water potential and the relative water content of the plants, which were maintained throughout the experimental period at approximately -0.25 MPa and 87.31%, respectively.

The electrolyte leakage in the development phase of the branches and leaves ranged from 29.39 to 26.33%, with no significant difference between DAD. Plants under the tensions of -10 , -40 and -70 kPa had similar electrolyte leakage (Fig. 5c).

The potential quantum efficiency of photosystem II of the plants was on average 0.75, independent of soil water tension. There was no difference between the plants subjected to different tensions (Fig. 5d). However, the effective quantum efficiency of photosystem II of the plants oscillated during the study phase, with a reduction of 57.4% at 45 DAD and an increase of 34.0% at 90 DAD. The plants under the tensions of -40 and -70 kPa had the effective quantum efficiency of photosystem II reduced by 13.3% because of water deficiencies (Fig. 5e).

The electron transport rate at 45 DAD was $90.43 \mu\text{mol m}^{-2} \text{s}^{-1}$, with an increase of 48.0% at 90 DAD (Fig. 5f). In general, lower soil water tensions (-40 and -70 kPa) caused a 16.2% reduction in the electron transport rate of cassava plants.

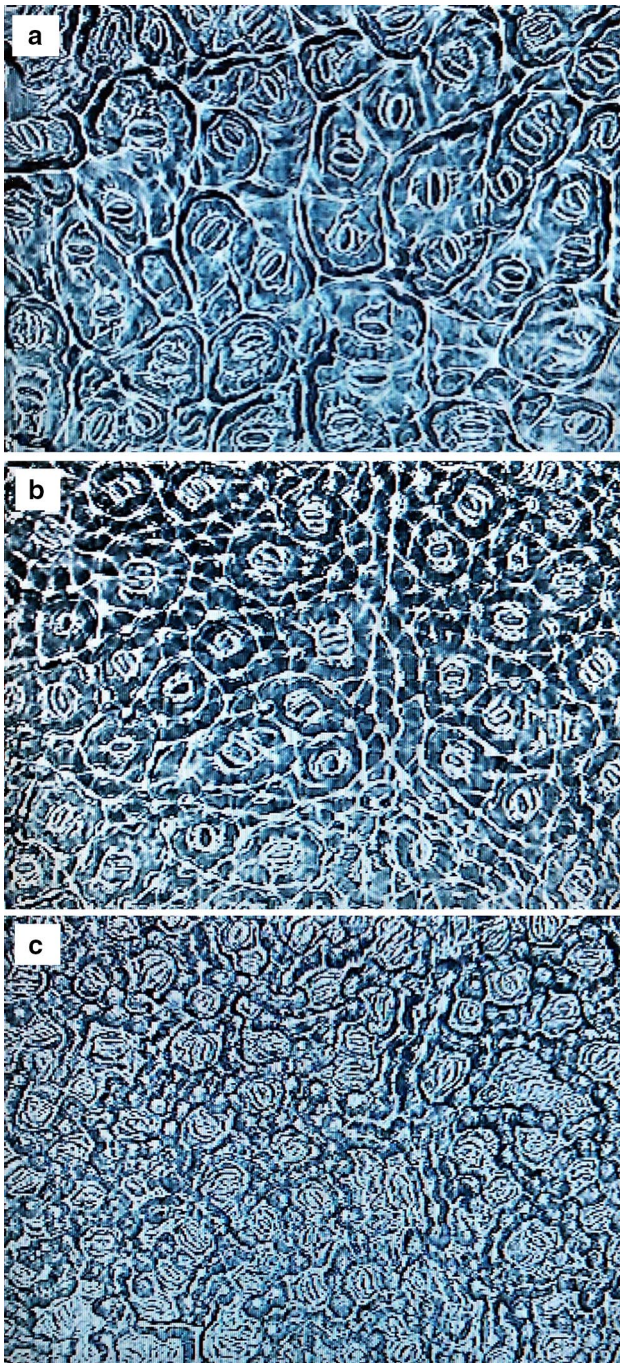


Fig. 4 Printing of the abaxial surface of the leaf in an area of 0.0744 mm^2 at 90 days of treatment under soil water tensions of -10 kPa (a), -40 kPa (b) and -70 kPa (c) in IAC 576-70 sweet cassava plants during the development phase of the branches and leaves

Discussion

The growth and production of cassava are favoured by average annual temperatures above $20 \text{ }^\circ\text{C}$ (El-Sharkawy 2003) and air humidities between 60 and 70% (Abreu et al. 2008).

The temperature and the relative humidity of the air inside the protected crop during the experimental phase were in the ideal range for the culture (Fig. 2a). Cassava plants are very sensitive to changes in atmospheric conditions. When exposed to dry air, the stomata rapidly close, reducing transpiration in VPD above 2 kPa . This mechanism of avoidance to stress maximizes the water use efficiency of photosynthesis, particularly under soil water deficiency, which is an advantage compared with less sensitive species such as maize (El-Sharkawy 2012).

Soil water tensions applied in the treatments of -40 and -70 kPa during the development phase of the branches and leaves of the sweet cassava IAC 576-70 (Fig. 2c) were reached approximately 15 days after the start of the treatments, which may have interfered in the physiological evaluations of the plants at 45 and 90 DAD because the water-deficit period corresponded to 30 and 75 DAD in these treatments, respectively.

The first response of plants to water deficiency is the reduction of stomatal conductance, which avoids loss of water to the atmosphere (Guo et al. 2010). However, the closure of the stomata has complex consequences that require adjustments in photosynthesis, respiration, nutrients and water flow (Bohnert and Jensen 1996). Photosynthesis is one of the main processes most affected by abiotic stress (Liu and Huang 2008).

Photosynthetic pigments are responsible for the capture of light energy and its conversion into chemical energy in the initial stages of photosynthesis. Chlorophyll *a* and *b* and carotenoids are located on the membranes of thylakoids in chloroplasts and constitute the most abundant natural pigment group present in plants (Taiz and Zeiger 2013). Chlorophylls are easily degraded because they have a chemically unstable structure (Streit et al. 2005). Therefore, photosynthetic pigments can be affected when a plant is subjected to dry conditions and can decrease the efficiency of light energy absorption, electron transfer and the production of ATP and NADPH in the photochemical phase of photosynthesis. Environmental factors such as water deficiency, luminosity, thermal changes, high levels of ethylene or a combination of factors may influence the degradation of chlorophyll (Heaton and Marangoni 1996).

A decrease in chlorophyll content when plants are subjected to water deficiency has been observed in diverse cultures such as wheat (Zaefyzadeh et al. 2009), sugarcane (Silva et al. 2013), sunflower (Manivannan et al. 2014), cotton (Shah et al. 2011) and maize (Miri and Armin 2013). However, in the present study, chlorophyll *a* and total chlorophyll content increased in plants under the lowest tension (-70 kPa), mainly at 45 DAD (Fig. 3a, d). This may have been a response of the acclimatization of cassava plants to water deficiency since the stomatal sensitivity of the crop is changed according to the climate and water in the soil

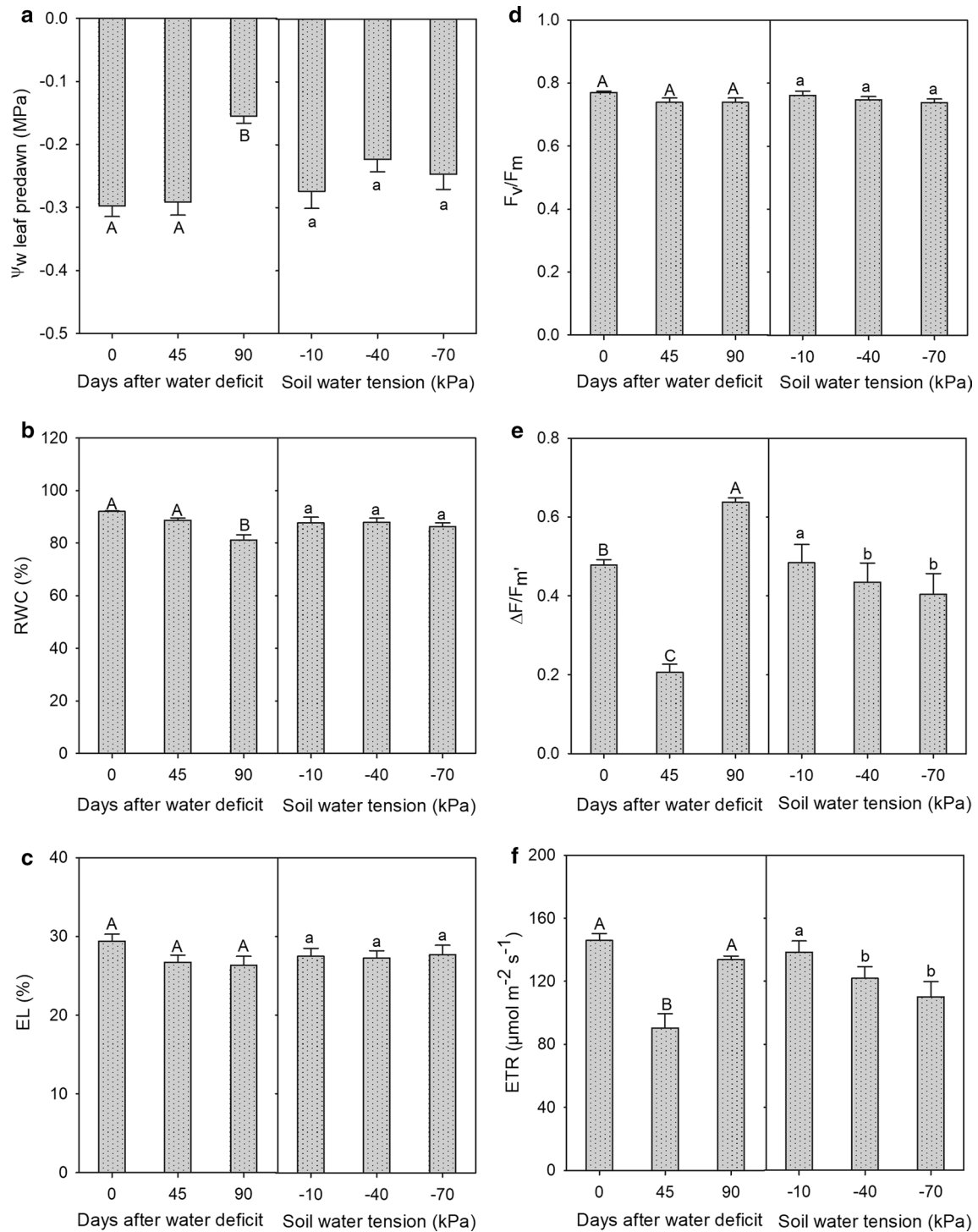


Fig. 5 Leaf water potential (**a**), relative water content (**b**), electrolyte leakage (**c**), potential quantum efficiency of photosystem II (**d**), effective quantum efficiency of photosystem II (**e**) and electron transport rate (**f**) in IAC 576-70 sweet cassava plants under different soil water tensions during the development phase of the branches and leaves.

Means followed by the same capital letter for the days and low-case letter for the tensions did not differ based on the Tukey test ($p \leq 0.05$). The bars indicate the standard error of the mean of five replicates

(El-Sharkawy 1993) and combines the heliotropic response of the leaves by folding them in the hottest hours of the day and opening them only when the VPD is smaller and the radiation is low (Calatayud et al. 2000), which could make photosynthesis difficult for most of the day. In addition to the verified foliar folding of the plants in the cultivar under study, the increase in chlorophyll content probably contributed to the more efficient absorption of the radiation when the stomatal conductance was higher.

Berg et al. (1986) studied cassava cultivated in the field and verified that the foliar folding phenomenon decreased leaf light interception by 50% and reduced leaf temperature from 6 to 7 °C and the VPD between leaf and air by 1.3 kPa while increasing the stomatal conductance twice as much as in leaves that remained horizontal. Therefore, leaf folding or falls at noon in irrigated or water-deficient plants may act as a mechanism to avoid water stress, conferring on cassava a prolonged drought tolerance response (El-Sharkawy 2004).

Carotenoids in photosynthetic tissues are protective pigments for chlorophyll protection against photooxidation (Bartley and Scolnik 1995). Cassava plants under a tension of – 70 kPa showed an increase in carotenoid contents at 45 DAD (Fig. 3e), which would certainly improve the dissipation of excess light energy in the antenna complex that was not being used in the photochemical phase of photosynthesis because of soil water deficiency. Pompelli et al. (2010) found an increase in the carotenoid contents in jatropha plants at 4 and 8 days of water deficit. Lin et al. (2006) studied sweet potato plants and did not observe differences in carotenoid contents between plants in control and water deficit at 14 days of treatment. In sugarcane, there was a decrease in carotenoid contents in plants submitted to 15 days of water deficit, but there were differences between cultivars (Santos et al. 2015). In rice, the decrease in carotenoids was reported by Usman et al. (2013). This suggests a great variability in crop behavior, since the responses to water deficiency depend on the interaction of several factors related to the plant and the environment.

Stomatal density is an important echo physiological parameter that affects gas exchange and photosynthesis (Camargo and Marengo 2011). Most of the cassava cultivars have many stomata on the abaxial leaf surface (300–500 mm⁻²) and practically none on the adaxial surface (El-Sharkawy et al. 1984). The abaxial surface of the cassava leaves has an epidermis with papilliform cells that add approximately 15% to the thickness of the leaf and increase the resistance to flow to the atmosphere by two to three times, which contributes to the conservation of water in the leaf while assimilating the CO₂ (Angelov et al. 1993).

Several cassava cultivars show plasticity in leaf anatomy and differences in stomatal characteristics, such as density, that can demonstrate potential cultivars for drought tolerance (Cerqueira 1992; El-Sharkawy 2006). The reduction

in the size of the stomata is an important event in the regulation of gas exchange since leaves with smaller stomata present a greater water use efficiency because they present a smaller size of the stomatal pores, thus conditioning a lower water loss through transpiration (Lake et al. 2001; Boeger and Wisniewski 2003). The tensions of – 40 and – 70 kPa promoted an increase in stomatal density on the abaxial surface of IAC 576-70 cassava leaves, which may be a response of acclimatization or adaptation of the plant to tolerate soil water deficiency. Other crops show foliar plasticity to changes of soil moisture status and dynamics. In potato, a reduction in leaf stomatal density was observed under severe water deficit, while under moderate water deficit, there were smaller and denser stomata production favoring gas exchange and optimization of water use efficiency (Sun et al. 2014). In maize, there was a significant increase in leaf stomatal density with soil water reduction, accompanied by decreased transpiration and net photosynthetic rate (Zhao et al. 2015). In wheat plants, the reduction of leaf stomatal density under water-deficit conditions was the main cause of increase in water use efficiency (Li et al. 2017). Changes in stomatal characteristics are associated by many authors, mainly regarding size and density, the adjustment of stomatal opening for better water use efficiency in response to atmospheric conditions (Yan et al. 2012; Egea et al. 2011; Zhang et al. 2006).

The water potential and the relative water content in the leaves are important characteristics that influence the water relations of the plants (Anjum et al. 2011). El-Sharkawy (2012) evaluated the response of cassava plants to environmental conditions and verified that stomata closure protected the leaf against dehydration without any changes in leaf water potential in plants with and without water deficiency. Tafur et al. (1997) did not observe significant differences in the water potential of cassava plants submitted to water deficiency and soil fertilization; they explained this result by stomatal closure and the ability of the plant to absorb water from the deep layers of the soil. In this study, cassava plants maintained the water potential and the relative water content unchanged in the predawn between plants under different soil water tensions (Fig. 5a, b). Alves and Setter (2004) studied cassava plants under water deficiency and concluded that the tolerance of the crop to drought can be attributed to the maintenance of the high-water potential of the tissues during the stress phase. Turyagyenda et al. (2013) investigated two cassava cultivars under 10 days of water deficit and verified differences in the relative water content of the leaves among the cultivars, although these did not differ from the plants in the control. In plants of tuberous roots such as sweet potato, maintenance of water potential in the tissues in adaptive response to drought has also been observed (Motsa et al. 2015). In tuber plants such as potato, the decrease stomatal

conductance occurs in parallel with the leaf water potential as a water conserving strategy (Obidiegwu et al. 2015). In bean, a similar result occurred with the imposition of the water deficit, with reductions in leaf water potential and stomatal conductance and consequent increase in intrinsic water use efficiency (Dias and Brüggemann 2010). However, maintaining cell turgescence has been attributed as a possible mechanism that confers drought tolerance on soybean (Ku et al. 2013; Bellaloui et al. 2013).

Cell membranes of leaf tissues are the first targets of many abiotic stresses (Levitt 1980). The extravasation of electrolytes in leaf fragments has been widely used as an estimate of injury caused by stresses in various cultures (Bajji et al. 2002; Zhao et al. 2009). Cassava plants showed cell membrane integrity independent of soil water status (Fig. 5c). Maintenance of membrane stability during severe water deficit is important to continue the physiological metabolism under low water potential (Tripathy et al. 2000). Faria et al. (2013) analyzed castor plants under water deficiency and observed that the extravasation of electrolytes differed between cultivars. A similar result was found in potato plants (Rudack et al. 2017). Differences in membrane integrity may be due to cell-wall properties and leaf structure (Kocheva et al. 2014). Wedeking et al. (2017) studied sugar beet under drought conditions and observed highly significant exponential correlations between temperature variation $\Delta T_{\text{air-leaf}}$ and membrane stability and leaf water status. Membrane injury index can be used as a useful parameter to select drought-tolerant genotypes (Ulemale et al. 2013).

The photosynthetic activity of plants can be evaluated by the analysis of chlorophyll *a* fluorescence. The potential quantum efficiency is presented by the F_v/F_m ratio determined after dark adaptation, where F_m is the maximum fluorescence and F_v is the variable fluorescence obtained by the difference between F_m and F_0 . F_0 refers to the initial fluorescence, corresponding to the emission of light by the excited chlorophyll molecules, before the energy is transmitted to the reaction centre of photosystem II (Rascher et al. 2000). The F_v/F_m ratio is used as a sensitive indicator of the photosynthetic performance of a plant and can range from 0.75 to 0.85 in vegetables under optimal environmental conditions. However, there is a decline in the potential quantum efficiency of photosystem II of a plant subjected to stressful environments, indicated by the fall in the ratio F_v/F_m (Björkman and Powles 1984; Krause and Weis 1991).

The effective quantum efficiency of photosystem II described by the $\Delta F/F_m'$ ratio, ΔF being the difference between the maximum fluorescence (F_m') and the dynamic equilibrium state fluorescence (F_s), both in the presence of light (Marques and Silva 2008), reveals the effectiveness of the use of the radiation absorbed by the chlorophylls associated with photosystem II through the efficiency in the electron transport, considered as indicative of photosynthesis.

Cassava plants did not show a decline in the F_v/F_m ratio when submitted to soil water deficiency (Fig. 5d), which indicates that the tensions of -40 and -70 kPa did not interfere in the maximum photosynthetic efficiency of the plants. On the other hand, the effective quantum efficiency of photosystem II and the electron transport rate of cassava plants were reduced under the lowest tensions (Fig. 5e, f). These changes were probably associated with photoinhibition because of super reduction of the electron acceptors in photosynthesis II (Foyer and Noctor 2000). An et al. (2015) researched the changes in chlorophyll fluorescence in cassava plants throughout the day and observed a positive correlation between the effective quantum efficiency of photosystem II and the rate of electron transport. Silva et al. (2010) evaluated the photosynthetic changes in jatropha plants caused by drought and heat and observed reductions of 56% in the effective quantum efficiency of photosystem II and 83% in the rate of electron transport; they did not present changes in the F_v/F_m ratio. In sweet potato, chlorophyll degradation in the leaves caused by induced drought contributed significant reductions in the effective quantum efficiency of photosystem II and in the F_v/F_m ratio at 3 and 4 months of treatment (Yooyongwech et al. 2017). In sugar beet, the efficiency of the photosynthetic apparatus declined under water-deficit conditions (Choluj et al. 2014).

From the results obtained, we conclude that a soil water tension of -70 kPa promotes an increase of the total chlorophyll content and carotenoids in IAC 576-70 sweet cassava plants at 45 days of water deficit. This cultivar can maintain a leaf water potential close to -0.3 MPa and a relative water content between 80 and 90% in the predawn and can maintain the integrity of cell membranes in the leaves and the potential quantum efficiency of photosystem II under soil water tensions of -10 , -40 and -70 kPa. Water deficiency impairs plant photosynthesis because of reductions in the effective quantum efficiency of photosystem II and the rate of electron transport; however, there is an increase of 16.0% in stomatal density in this stress condition.

Author contribution statement LFMP: set up and carried out the experiment, analyzed and interpreted the data, and prepared the manuscript. SZ: set up and carried out the experiment. MAS: planned and wrote the proposal, designed and supervised the experiment, interpreted the data, and was responsible for the manuscript revision.

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