Methyl jasmonate modulation reduces photosynthesis and induces synthesis of phenolic compounds in sweet potatoes subjected to drought

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ABSTRACT: Sweet potato [*Ipomoea batatas* (L.) Lam.] has wide adaptability to different climatic conditions. However, its yield can be affected by prolonged periods of drought. Application of exogenous jasmonates can modulate several physiological and biochemical processes, improving plant tolerance to abiotic stress. This study sought to evaluate the role of exogenous application of methyl jasmonate (MeJA) in attenuating the adverse effects of drought stress by physiobiochemical analyses and their impact during the early initiation of tuberous roots. The experimental design was completely randomized and arranged in a 2 × 2 factorial, comprised of two concentrations of a MeJA plant regulator [without (0 µmol·L⁻¹) and with (13 µmol·L⁻¹) application] and two water regimes (optimum and drought conditions, corresponding to a field capacity of 100 and 40%, respectively). Plants treated with MeJA showed a reduction in total leaf area and leaf dry biomass but increased adventitious root dry biomass. In addition, MeJA application in sweet potato plants affected photosynthetic performance and increased and antioxidant phenolic compounds, carotenoids, anthocyanins, and proline. The evaluated response mechanisms showed that the severity of drought was more prominent than the positive effects of MeJA, since the increases on antioxidant pigments and secondary metabolites were not sufficient to mitigate stress caused by drought, which was reflected in the reduced tuberous root production.

Key words: photosynthetic performance, phenylalanine ammonia lyase, tuberous roots, *Ipomoea batatas* (L.).

INTRODUCTION

Ipomoea batatas (L.) Lam. (sweet potato) is a starchy tuberous root containing several vitamins, minerals, and proteins (Shigematsu et al. 2017), which is why it is one of the seven major food crops cultivated worldwide (Li et al. 2018). Recent research has reported that sweet potato, when included in the human diet, is beneficial for preventing many diseases (Esatbeyoglu et al. 2017), making it a functional food. This is due to high contents of polyphenolic compounds and carotenoids (Albishi et al. 2013; Esatbeyoglu et al. 2017; Wang et al. 2018).

Despite its rusticity, easy maintenance, short crop cycle, resistance to diseases and pests, wide adaptation to arid and dry regions, and high yield potential, sweet potato yield is affected in regions exposed to strict drought regimes (Mbinda et al. 2016; 2018), particularly during the establishment phase, including early vine development and storage root initiation

(Gajanayake et al. 2014). The formation of tuberous roots in sweet potatoes can begin within four weeks after planting the branch, depending on the cultivar and environmental conditions. In this phase it is ideal to have favorable conditions in terms of soil moisture and temperature (Gajanayake and Reddy 2016).

Jasmonic acid and its methyl ester methyl jasmonate (MeJA) are considered plant regulators that occur naturally in plants and control morphological, physiological, and biochemical processes (Ueda and Saniewski 2006; Norastehnia et al. 2007). Both are involved in signal transduction pathways in plant responses to environmental stressors. The exogenous application of jasmonates can modulate several physiological responses that lead to increased resistance to abiotic stress (Walia et al. 2007). The application of plant regulators such as salicylic acid, MeJA, and abscisic acid in sweet potato increases the levels of antioxidant compounds, including phenolics, flavonoids, anthocyanins, and β-carotene (Ghasemzadeh et al. 2016). Plants exhibiting increased synthesis of polyphenols under abiotic stress usually show better adaptability to limiting environments, since these compounds have antioxidative properties and are capable of scavenging free radicals, protecting plant cells from negative effects of oxidative stress (Sharma et al. 2016).

The hypothesis of this work is that the exogenous application of MeJA modulates the protection mechanisms against drought, increasing production of phenolic compounds in sweet potato leaves and roots. Thus, this study sought to evaluate the role of exogenous application of MeJA in attenuating the adverse effects of drought stress by physiobiochemical analyses and their impact during the early initiation of tuberous roots.

MATERIAL AND METHODS

Experimental site

The experiment was carried out at the University of Western São Paulo (UNOESTE), Presidente Prudente, State of São Paulo, Brazil (22°06'59" S and 51°27'12" W; 402 m above sea level). The experiment was conducted in a semicontrolled greenhouse environment (temperature and humidity), between November 2018 and January 2019.

Design and experimental treatments

The experimental design was completely randomized with 10 replications and arranged in a 2 × 2 factorial scheme, comprising two concentrations of a MeJA plant regulator [without (0 μmol·L^{-1}) and with (13 μmol·L^{-1}) application] in combination with two water regimes (optimum and drought conditions, which correspond to field capacity at 100 and 40%, respectively). The plots were composed of 40 pots with one plant each. Twenty-four pots were used for photosynthetic, biochemical, biometric and yield evaluation. The remaining 16 pots were used to evaluate leaf water potential and discarded at the end of the experiment.

Cultivation conditions

A mixed soil of dystrophic ultisol (Santos et al. 2018) was used with a Carolina - XVI substrate, in the ratio 2:1, respectively. The Carolina - XVI substrate used was composed of peat, vermiculite, and limestone.

The soil was collected at a 0.0–0.2 m depth. It was crumbled, air-dried and sieved (4.0 mm) and shown the following chemical attributes: pH ($CaCl_2$) 4.2, organic matter 3 g·dm^{-3}, P (resin) 3 mg·dm^{-3}, K 1.36 $mmol_c$·dm^{-3}, Ca 4.53 $mmol_c$·dm^{-3}, Mg 3.26 $mmol_c$·dm^{-3}, S 10.73 mg·dm^{-3}, B 0.29 mg·dm^{-3}, Cu 0.6 mg·dm^{-3}, Fe 2.97 mg·dm^{-3}, Mn 1.13 mg·dm^{-3}, Zn 0.23 mg·dm^{-3}, potential acidity (H + Al) 21.83 $mmol_c$·dm^{-3}, Al 7.97 $mmol_c$·dm^{-3}, sum of bases 9.15 $mmol_c$·dm^{-3}, cation exchange capacity 30.98 $mmol_c$·dm^{-3}, and base saturation 29.5%. Based on the chemical analysis, soil liming was carried out to raise the base saturation to 70% (Quaggio et al. 1985), by the addition of 750 mg·dm^{-3} dolomitic limestone (36% $CaCO_3$ and 15.8% $MgCO_3$). The soil containing carbonate salts was incubated for 30 days in pots at a humidity of 80% at field capacity to allow it to equilibrate.

Experimental process

Fertilization was carried out with 1 mg·dm^{-3} of the formulation 4-30-10 (N-P-K). After 7 days, slips of sweet potato cultivar Beauregard (20 ± 1 cm) were transplanted to pots (one slip per pot) with a capacity of 8 dm^3 at a depth of 10 cm, which were cultivated for a period of 5 weeks for adventitious root formation. Cover fertilization with 0.42 mg·dm^{-3} of the formulation 20-05-20 (N-P-K) was done in the 4th week.

After the initial acclimatization period, plants were standardized for length of the branch (approximately 40 ± 1 cm in length) and number of leaves (approximately 12 leaves per branch), selecting those with greater vigor and homogeneity of size.

MeJA application and water deficit imposition

Before subjecting the plants to drought, field capacity of the soil [100% water mass (g) that the soil supports] was determined. The water deficit was imposed 37 days after slip transplantation, coincided with the tuberization start period. Plants remain in this condition for 9 days (Yooyongwech et al. 2013), that is, until 46 days after slip transplantation, with a daily replacement up to 40% of field capacity, based on Gajanayake and Reddy (2016). Under optimum water conditions, plants were watered daily to field capacity. During this period, the mass of each pot was measured once a day, in the morning, and the volume of water lost by evapotranspiration was restored.

Methyl jasmonate application (224.30 MW, Sigma) was performed at a concentration of 13 μmol·L^{-1} (Ghasemzadeh et al. 2016). Methyl jasmonate application was performed via manual foliar pulverization one day before and on the 5th day of water deficit imposition (50 mL of the solution per plant).

Measurements of gas exchange

On the 3rd, 6th, and 9th day of water deficit imposition, gas exchange evaluations were carried out using a portable infrared gas analyzer (IRGA, Li-6400XTR, LiCor, EUA) by choosing two leaves from each plant between the sixth and ninth fully developed leaf of the branch. Evaluations were carried out on a clear day between 10:00 a.m. and 12:00 p.m. Photosynthetically active radiation (PAR) was standardized to an artificial saturating light of 1200 μmol·m^{-2}·s^{-1} and the concentration of CO_2 was established at 380 ± 10 μmol·mol^{-1}. The average relative humidity, the temperature and the vapor pressure deficit were 50%, 26 ± 2 °C and 2.22 ± 0.47 kPa, respectively.

The net photosynthetic rate (A, μmol CO_2·m^{-2}·s^{-1}), stomatal conductance (gs, mol H_2O·m^{-2}·s^{-1}), internal concentration of CO_2 in the substomatic chamber (Ci, μmol CO_2 mol·ar^{-1}), transpiration rate (E, mmol H_2O·m^{-2}·s^{-1}), water use efficiency [WUE, (A/E) μmol CO_2·mmol^{-1} H_2O], and instantaneous carboxylation efficiency [EiC, (A/Ci)mol·air^{-1}] were obtained.

Measurements of leaf water potential (Ψw)

The evaluation days were the same as those described in gas exchange measurements. Two leaves from each plant were chosen between the sixth and ninth fully developed leaf of the branch and measurements were recorded at 12:00 p.m. Measurements were made in a pressure chamber (model 1000, PMS Instruments, USA), expressed in MPa (Scholander et al. 1965).

Material storage for biochemical analysis

46 days after slip transplantation, leaves were collected between the sixth and ninth fully developed leaf of the branch (counting down from the apex) of each plant and immediately immersed in liquid N$_2$ for rapid freezing. The material was stored at −80 °C for further analysis.

Analysis of photosynthetic and antioxidant pigments

Methods for determining chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Tchl), and carotenoid contents (CAR) were based on those described by Hiscox and Israelstam (1979). Fresh leaf tissue (0.1 g) was incubated in a water bath at 65 °C for 1 h containing 7 mL of DMSO. After that time, the samples were cooled in the dark until they reached room temperature. The readings were performed in a spectrophotometer at 663, 645 and 480 nm. Photosynthetic pigment content was calculated following the equation used by Arnon (1949) and expressed in $\mu g \cdot g^{-1}$ FW.

Anthocyanins were determined according to Francis (1982). Fresh leaf tissue (1 g) was macerated in a 95% ethanol extract solution acidified with 1.5 N HCl and stayed for 24 h at a temperature of 5 °C. The absorbance was read at 535 nm using a spectrophotometer (BEL Engineering, model UV-M51) and the resulted was expressed in $\mu g \cdot 100^{-1}$ g FW.

For determination of the β-carotene content, fresh (leaves and roots) tissue (5 g) were ground and packed in a volumetric flask to protect from light. Then, 50 mL of a 2:1:1 hexane, acetone, and ethanol mixture was added to solubilize the carotenoids (Sadler et al. 1990). The samples were stirred for 30 min and 10 mL of distilled water was added. The solution was allowed to separate into a distinct polar layer (35 mL) and a nonpolar layer (25 mL). The absorbance was read at 450 nm using a spectrophotometer and expressed in $\mu g \cdot g^{-1}$ FW, calculated according to the equation by Craft and Soares Junior (1992).

Polyphenolic compounds and phenylalanine ammonia lyase enzyme activity (PAL, EC 4.3.1.5) assay

The ethanolic crude extracts obtained were performed according to the method by Simões et al. (2007). Total polyphenolic content of leaves (TPL) and roots (TPR) were determined according to the Folin–Ciocalteau reagent method. The samples (25 μL) were mixed with 125 μL of Folin–Ciocalteau reagent, 350 μL of 25% sodium carbonate solution and 2 mL of water (Stagos et al. 2012). The mixture was incubated for 1 h at room temperature. Absorbance was read at 765 nm and the result was expressed in $\mu g \cdot mL^{-1}$ of gallic acid equivalent (GAE). The total flavonoid content of leaves (TFL) and roots (TFR) was measured according to the method by Yao et al. (2013). The samples (100 μL) were mixed with 400 μL of ethanol 70%, 50 μL of $NaNO_2$ (5%), 50 μL of $AlCl_3$ (10%), 300 μL of NaOH (1 $mol \cdot L^{-1}$) and 100 μL of water. The mixture was incubated for 15 minutes in the dark. Absorbance was read at 510 nm and the result was expressed in $\mu g \cdot mL^{-1}$ of rutin equivalent (RE).

The enzymatic activity of PAL (EC 4.3.1.5) was evaluated according to Hyodo et al. (1978). Phenylalanine ammonia lyase enzyme activity was assayed by following (E)-cinnamic acid formation at 290 nm in a spectrophotometer at 40 °C in buffer (0.5 M TRIS-EDTA, either pH 8.5) containing 30 $\mu mol \cdot L^{-1}$ L-phenylalanine. A molar extinction coefficient of 104 $mmol \cdot L^{-1} \cdot cm^{-1}$ (Zucker 1965) was used for calculation. The results were expressed in $Kat \cdot sec^{-1} \cdot mg^{-1}$ protein. The protein content was determined, as described by Bradford (1976), using bovine serum albumin as a standard.

Malondialdehyde (MDA) and leaf proline content evaluation

Lipid peroxidation was determined by the production of 2-thiobarbituric acid (TBA)-reactive substances, especially MDA, according to Heath and Packer (1968). Fresh leaf tissue (0.25 g) was ground in liquid N_2 with a pestle and mortar, to which 3 mL of 0.1% trichloroacetic acid (TCA) in 20% polyvinyl polypyrrolidone (PVPP) was added. After complete homogenization, the samples were centrifuged at 10,000 rpm for 10 min at 4 °C. 0.25 mL of supernatant was added to 1 mL 20% TCA solution containing 0.5% thiobarbituric acid (TBA). The samples were kept in a dry bath at 95 °C for 30 min and then on ice for 20 min. Subsequently, the samples were centrifuged at 10,000 rpm for 5 min. Samples were read at two wavelengths, 535 and 600 nm and the resulted was expressed as $nmol \cdot g^{-1}$ FW.

Proline content in leaf tissues was measured via reaction with ninhydrin (Bates et al. 1973). Fresh leaf tissue (0.5 g) was ground in 5 mL of 3% sulphosalicylic acid and centrifuged at 13,000 rpm for 10 min at 4 °C. Two milliliters of supernatant was incubated with equal volume of acid ninhydrin and glacial acetic acid at 100 °C for 1 h. The reaction mixture was extracted with 2 mL toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was read at 520 nm with a spectrometer using L-proline as a standard. Proline content was expressed as $\mu mol \cdot g^{-1}$ FW.

Partitioned biomass production

46 days after slip transplantation, plants were separated into the shoot (leaves and stems) and roots (adventitious and tuberous). Roots with a diameter equal to or greater than 5 mm were considered tuberous roots (Villordon et al. 2009). Total leaf area (TLA, cm^2) was measured using a portable area meter (model LI - 3000A, LI-COR, USA). The number of tuberous roots (NTR) and diameter of the tuberous roots (DTR) was determined with a digital caliper and were expressed in mm. Subsequently, the material was placed in an oven with air circulation at 65 °C for 72 h to measure the leaf (LDB), steam (SDB), total shoot (TSDB), tuberous root (TUDB), adventitious root (ARDB), and total root (TRDB) dry biomass, expressed in g·plant^{-1}.

Statistical analysis

In all considered datasets, normality of the data was analyzed using the Anderson–Darling test and homoscedasticity of the data was verified with Levenn's test, both at 0.05 probability. Data were subjected to analysis of variance (ANOVA) using the F test ($p \leq 0.05$). When significant, the traits were subjected to the Tukey's test ($p < 0.05$). All statistical analysis of the data was performed using protocols developed in the R software (R Development Core Team 2019).

RESULTS

Gas exchange and leaf water potential

On the 3rd, 6th, and 9th day, all gas exchange traits and Ψw showed an isolated effect for drought (Table S1). On these same days, A and Ci showed an isolated effect for MeJA, while the E showed a significant effect only on the 6th and 9th day. Plants cultivated under drought showed reductions in A, gs, Ci, E, and Ψw on the 3rd, 6th, and 9th day (Table 1).

Plants treated with MeJA decreased A by 15, 22, and 30% and increased Ci by 15, 9, and 4%, on the 3rd, 6th, and 9th day, respectively. The gs and Ψw were unchanged in plants treated with MeJA on the 3rd, 6th, or 9th day. The same behavior was observed in E, but only on the 3rd day. On the 6th and 9th day, E showed a reduction of 18% (Table 1).

Table 1. Results for CO_2 assimilation rate (A, µmol CO_2·m^{-2}·s^{-1}), stomatal conductance (gs, µmol CO_2·m^{-2}·s^{-1}), internal CO_2 concentration in the substomatic chamber (Ci, µmol CO_2·m^{-2}·air^{-1}), transpiration rate (E, mmol H_2O·m^{-2}·s^{-1}), and water potential (Ψw, MPa) evaluated on the 3rd, 6th, and 9th day in sweet potato 'Beauregard' treated with two concentrations of a MeJA plant regulator [without (0 µmol·L^{-1}) and with (13 µmol·L^{-1}) application] in combination with two water regimes (optimum and drought conditions, which correspond to field capacity at 100 and 40%, respectively).

Days of stress	Factors	Levels	A	gs	Ci	E	Ψw
3rd	Water regime	Optimum	23.71a	0.79a	373.28a	11.06a	-0.55a
		Drought	0.81b	0.02b	304.01b	0.63b	-0.90b
	MeJA	Without	13.23A	0.42	315.09B	5.98	-0.73
		With	11.29B	0.39	362.20A	5.72	-0.71
6th	Water regime	Optimum	27.42a	0.78a	353.20a	9.10a	-0.50a
		Drought	2.56b	0.07b	298.35b	1.23b	-0.94b
	MeJA	Without	13.23A	0.42	315.09B	5.98	-0.73
		With	11.29B	0.39	362.20A	5.72	-0.71
9th	Water regime	Optimum	22.18a	0.67a	315.06a	9.28a	-0.52a
		Drought	3.30b	0.05b	292.00b	0.89b	-0.92b
	MeJA	Without	15.00A	0.44	297.00B	5.58A	-0.71
		With	10.47B	0.27	310.06A	4.59B	-0.73

Lowercase letters compare water regimes independent of MeJA, while uppercase letters compare the effect of MeJA independent of the water regime.

An interaction for water regime and MeJA application was observed in *EiC* on the 3rd and 6th day and in *WUE* on 6th day (Table S1). Plants subjected to drought showed reductions in *EiC* on the 3rd, 6th, and 9th day. Water use efficiency decreased on the 3rd day, but on the 6th day the decrease was only observed in plants treated with MeJA. Conversely, *WUE* increased on the 9th day (Table 2).

In relation to MeJA application, on the 3rd, 6th, and 9th day, plants showed a reduction in *EiC*, except under drought on the 3rd day. Water use efficiency was unchanged in plants treated with MeJA on the 3rd day. This behavior was also observed in plants under optimum water conditions on the 6th day. Plants treated with MeJA and cultivated under drought showed a decrease in *WUE* on the 6th. A decrease in *WUE* was also observed on the 9th day, however this effect was independent of water regime (Table 2).

Table 2. Result for instantaneous carboxylation efficiency (*EiC*, mol·air^{-1}) and water use efficiency (*WUE*, µmol CO_2·mmol^{-1} H_2O) evaluated on the 3rd, 6th, and 9th day in sweet potato 'Beauregard' treated with two concentrations of a MeJA plant regulator [without (0 µmol·L^{-1}) and with (13 µmol·L^{-1}) application] in combination with two water regimes (optimum and drought conditions, which correspond to field capacity at 100 and 40%, respectively).

Days of stress	Water regime	EiC			WUE		
		MeJA		Average	MeJA		Average
		Without	With		Without	With	
3rd	Optimum	0.07Aa	0.06Ba	0.06	2.25	2.04	2.14a
	Drought	0.00Ab	0.00Ab	0.00	1.04	1.16	1.10b
	Average	0.04	0.03		1.65	1.60	
6th	Optimum	0.09Aa	0.06Ba	0.07	3.03Aa	3.05Aa	3.04
	Drought	0.01Ab	0.00Bb	0.01	2.85Aa	1.38Bb	2.11
	Average	0.05	0.03		2.94	2.21	
9th	Optimum	0.08	0.05	0.07a	2.57	2.27	2.42b
	Drought	0.02	0.00	0.01b	4.70	2.67	3.68a
	Average	0.05A	0.03B		3.63A	2.47B	

Lowercase letters compare the effect of MeJA between different water regimes (columns), while uppercase letters compare the effect of MeJA on the same water regime (rows).

Photosynthetic and antioxidant pigments

All traits (Chl *a*, Chl *b*, Tchl, and CAR) presented in Fig. 1 showed an interaction between water regime and MeJA application (Table S2). Plants cultivated under drought presented marked reductions in Chl *a*, Chl *b*, Tchl, and CAR (Fig. 1).

Plants treated with MeJA showed reduced Chl *a* (9 and 11%), Tchl (6 and 9%), and CAR (29 and 3%), when cultivated under optimum water conditions or drought, respectively (Figs. 1a and 1c-d). The Chl *b* was less sensitive and reduced only under drought, decreasing by 3% (Fig. 1b).

When comparing the behavior of anthocyanins and β-carotene between water regimes, it was observed that plants under drought showed the lowest TA content (Fig. 2a). Conversely, Lβ-car and Rβ-car increased in this condition of water supply, with the exception of Lβ-car in plants not treated with MeJA (Figs. 2b-c).

Plants treated with MeJA increased TA by 9% and 10% and Rβ-car by 110 and 9%, when cultivated under optimum water conditions and drought, respectively (Figs. 2a and 2c). The same behavior was observed on Lβ-car in plants cultivated under drought, registering an increase of 12% (Fig. 2b). However, plants cultivated under optimum water conditions showed the opposite behavior, that is, Lβ-car was reduced by 13% when MeJA was applied (Fig. 2b).

Phenolic compounds and oxidative stress

All traits (TPL, TPR, TFL, TFR, PAL, MDA, and proline) presented in Figs. 3 and 4 showed an interaction between the water regime and application of MeJA (Table S3. Supplementary material). Plants cultivated under drought showed
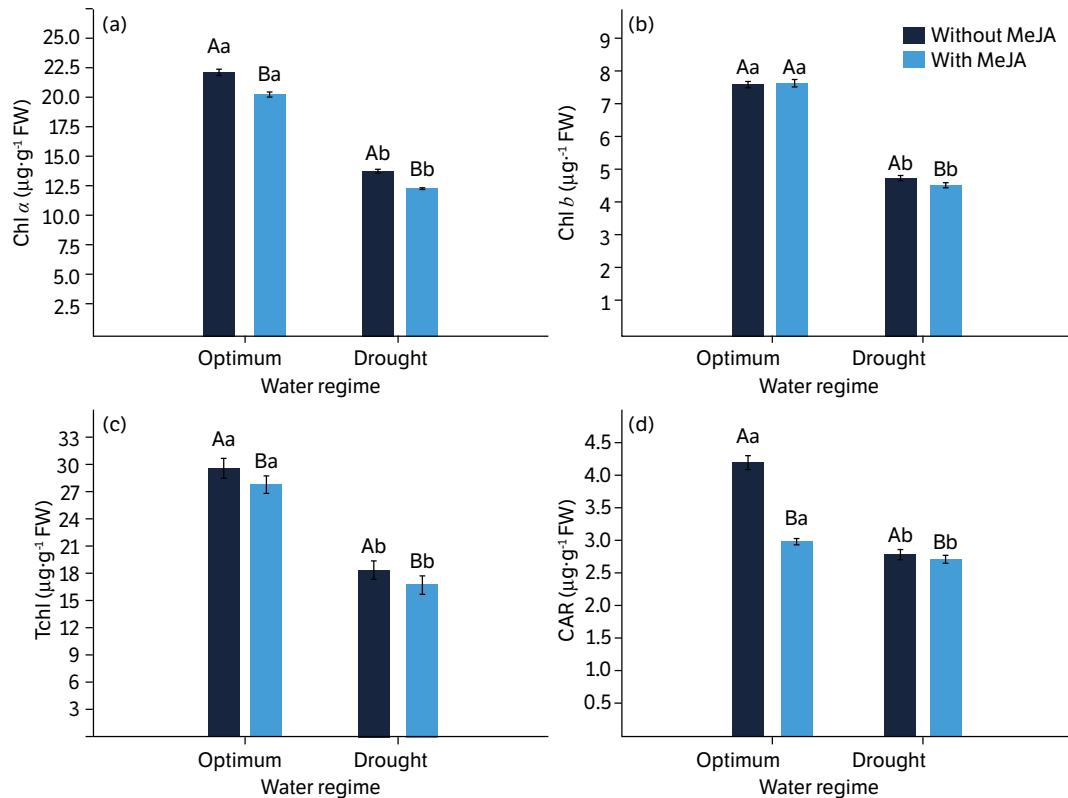
Figure 1. Chlorophyll *a* (A, Chl *a*), chlorophyll *b* (B, Chl *b*), total chlorophyll (C, Tchl), and carotenoids (D, CAR) on the 9th day in sweet potato 'Beauregard' treated with two concentrations of a MeJA plant regulator [without (0 μmol·L^{-1}) and with (13 μmol·L^{-1}) application] in combination with two water regimes (optimum and drought conditions, which correspond to field capacity at 100 and 40%, respectively). Lowercase letters compare the effect of MeJA between the water regimes, while uppercase letters compare the MeJA effect on the same water regime. Vertical bars represent the standard error.
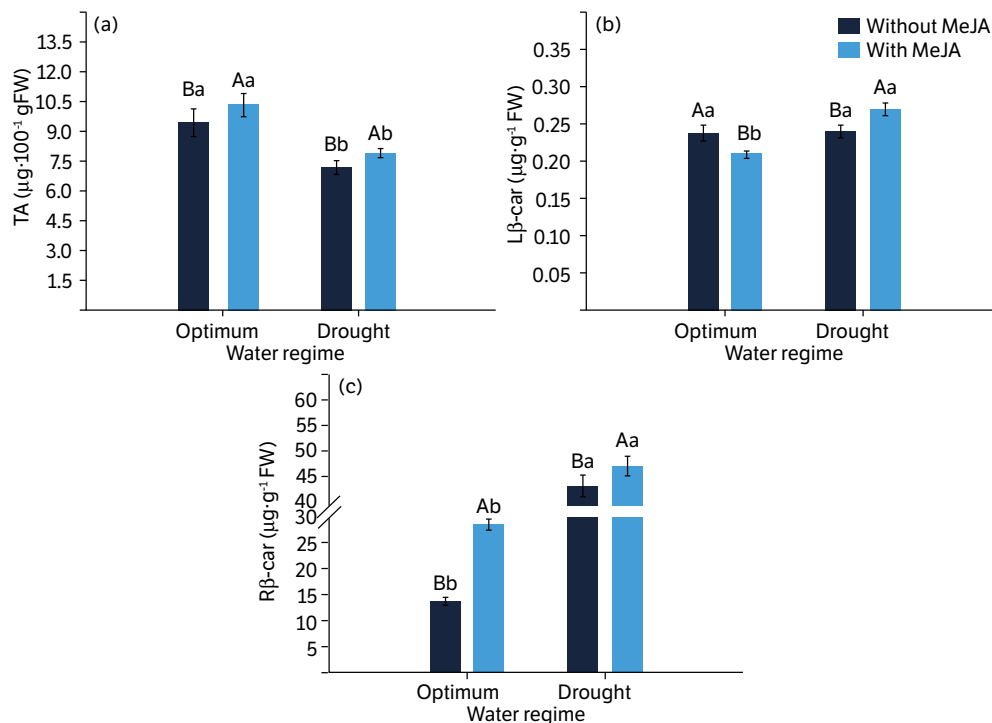
Figure 2. Total anthocyanin (TA, A), leaf β-carotene (Lβ-car, B) and root β-carotene (Rβ-car, C) on the 9th day in sweet potato 'Beauregard' treated with two concentrations of a MeJA plant regulator [without (0 μmol·L^{-1}) and with (13 μmol·L^{-1}) application] in combination with two water regimes (optimum and drought conditions, which correspond to field capacity at 100 and 40%, respectively. Lowercase letters compare the effect of MeJA between the water regimes, while uppercase letters compare the MeJA effect on the same water regime. Vertical bars represent the standard error.
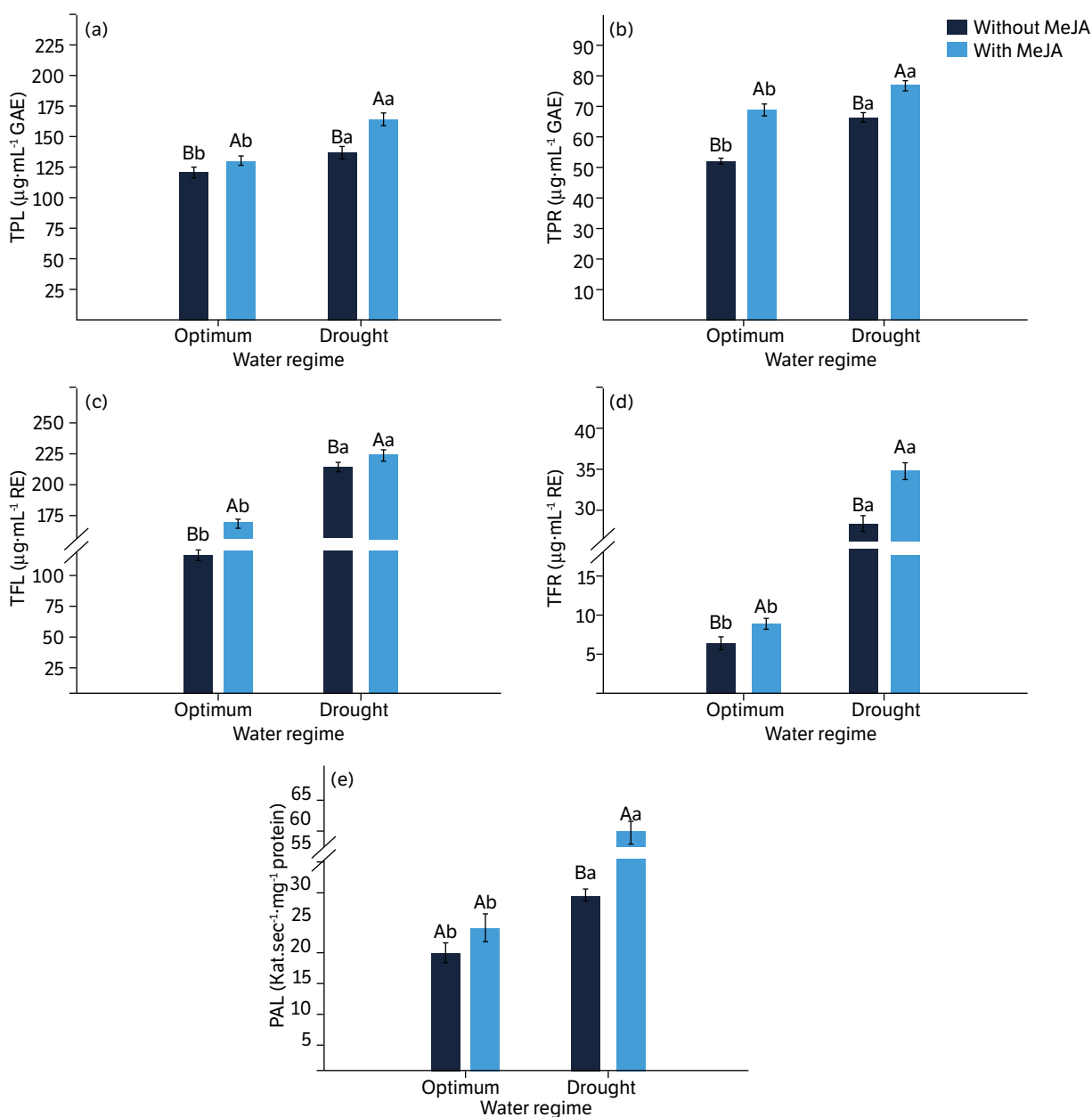
Figure 3. Total polyphenolic in leaf (TPL, A), total polyphenolic in root (TPR, B), total flavonoid in leaf (TFL, C), total flavonoid in root (TFR, D) and activity of the enzyme phenylalanine ammonia lyase (PAL, E) on the 9th day in sweet potato 'Beauregard' treated with two concentrations of a MeJA plant regulator [without (0 µmol·L^{-1}) and with (13 µmol·L^{-1}) application] in combination with two water regimes (optimum and drought conditions, which correspond to field capacity at 100 and 40%, respectively). Lowercase letters compare the effect of MeJA between the water regimes, while uppercase letters compare the MeJA effect on the same water regime. Vertical bars represent the standard error.

the highest content of TPL, TPR, TFL, and TFR. MeJA application increased the content of these traits, in both water regimes (Figs. 3a-d). Phenylalanine ammonia lyase enzyme activity showed this same behavior, except for plants treated with MeJA when cultivated under optimum water conditions (Fig. 3e).

Plants cultivated under drought showed the highest MDA content (Fig. 4a). Malondialdehyde was lower in plants treated with MeJA, in optimum conditions (-50%) and under drought stress (-54%). Plants cultivated under drought conditions and treated with MeJA had an increased proline content (Fig. 4b).
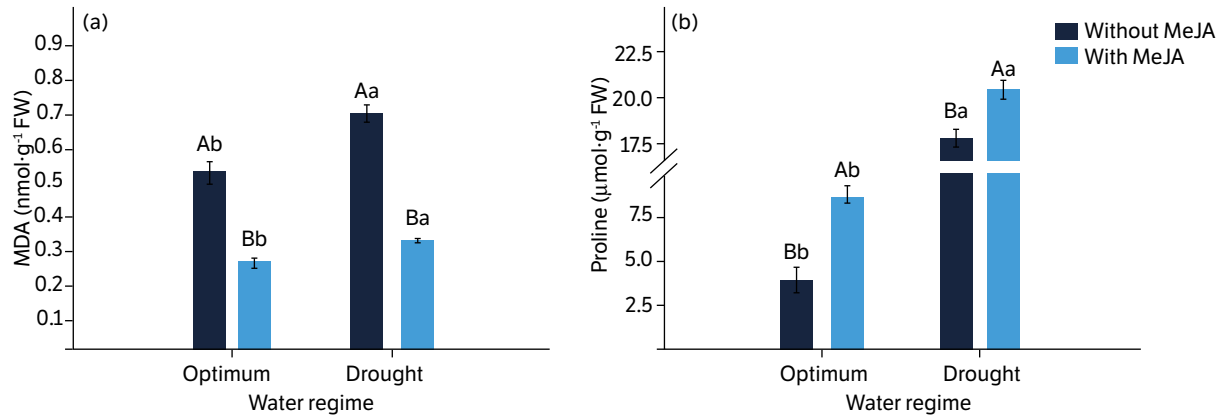
Figure 4. Malondialdehyde (MDA, A) and proline (B) on the 9th day in sweet potato 'Beauregard' treated with two concentrations of a MeJA plant regulator [without (0 µmol·L⁻¹) and with (13 µmol·L⁻¹) application] in combination with two water regimes (optimum and drought conditions, which correspond to field capacity at 100 and 40%, respectively). Lowercase letters compare the effect of MeJA between the water regimes, while uppercase letters compare the MeJA effect on the same water regime. Vertical bars represent the standard error.

Partitioned biomass production

The TLA and LDB parameters showed an isolated effect for water regime and MeJA application. The NTU, DTU, SDB, TSDB, TUDB, and TRDB parameters showed an isolated effect for water regime and an effect for MeJA application was detected on ARDB (Table S4). Plants cultivated under drought showed reductions in TLA, NTU, and DTU. Plants treated with MeJA reduced LA by 6%. (Table 3).

Plants cultivated under drought showed reductions in all assessed dry biomass traits (LDB, SDB, TSDB, TUDB, and TRDB). Plants treated with MeJA showed a reduction of 12% in LDB; in contrast, there was an increase of 12% in ARDB (Table 4).

Table 3. Result for total leaf area (TLA, cm²), number of tuberous root (NTU, unity·plant⁻¹) and diameter of tuberous root (DTU, cm) evaluated on the 9th day in sweet potato 'Beauregard' treated with two concentrations of a MeJA plant regulator [without (0 µmol·L⁻¹) and with (13 µmol·L⁻¹) application] in combination with two water regimes (optimum and drought conditions, which correspond to field capacity at 100 and 40%, respectively).

Factors	Levels	TLA	NTU	DTU
Water regime	Optimum	5813.82a	19.75a	1.45a
	Drought	2400.46b	17.45b	1.19b
MeJA	Without	4223.99A	19.15A	1.35A
	With	3990.29B	18.05A	1.30A

Lowercase letters compare water regimes independent of MeJA, while uppercase letters compare the effect of MeJA independent of the water regime.

Table 4. Tukey's test result for leaf (LDB, g·plant⁻¹), steam (SDB, g·plant⁻¹) total shoot (TSDB, g·plant⁻¹), tuberous root (TUDB, g·plant⁻¹), adventitious root (ARDB, g·plant⁻¹) and total root dry biomass (TRDB, g·plant⁻¹) evaluated on the 9th day in sweet potato 'Beauregard' treated with two concentrations of a MeJA plant regulator [without (0 µmol·L⁻¹) and with (13 µmol·L⁻¹) application] in combination with two water regimes (optimum and drought conditions, which correspond to field capacity at 100 and 40%, respectively).

Factors	Levels	LDB	SDB	TSDB	TUDB	ARDB	TRDB
Water regime	Optimum	18.50a	25.70a	44.2a	67.36a	33.43a	100.80a
	Drought	9.50b	17.40b	26.9b	48.64b	35.45a	84.10b
MeJA	Without	14.90A	22.30A	37.90A	61.05A	31.23B	92.20A
	With	13.10B	20.80A	33.90A	54.96A	37.64A	92.60A

Lowercase letters compare water regimes independent of MeJA, while uppercase letters compare the effect of MeJA independent of the water regime.

DISCUSSION

Gas exchanges and water potential

In plants treated with MeJA, reductions were observed in A, however gs was not altered, which shows that the reduction in A was due to cumulative limitations in carboxylation reactions (i.e., biochemical limitations), as verified by the lower EiC (Tables 1 and 2). According to Jung (2004) and Springer et al. (2015), jasmonic acid and MeJA applied exogenously led to decreased expression of genes related to photosynthesis, such as the gene encoding the small subunit of ribulose-1.5-bisphosphate carboxylase/oxygenase (Rubisco). The reduction in translation and increase in the degradation of Rubisco were accompanied by a rapid loss of chlorophyll in barley leaves (Weidhase et al. 1987). There was also a reduction in WUE on the 9th day of water deficit imposition, which is due to a substantial reduction in A (Tables 1 and 2).

Traits A, gs, Ci, and E showed decreases under drought. The decrease in gs is an adaptive behavior of the plant to prevent dehydration of the leaf tissue, which negatively impacted A, Ci and E (Table 2). Even under stomatal limitation, the Ψw continued to maintain dehydration levels, indicating that the water status of sweet potato plants cultivated under drought was affected. Similar results were observed by Gajanayake and Reddy (2016). These authors, studying irrigation depths based on the replacement of water lost by evapotranspiration (100, 60, 40, and 20%), verified reductions in A, gs, and E in sweet potato 'Beauregard'. Plants exposed to drought also showed a reduction in EiC (Table 3). Thus, the decrease observed in A is linked to stomatal and biochemical limitations (Tables 2 and 3).

Photosynthetic pigments

The photosystems in plants are composed of a core complex (Chl a and β-carotene) and a peripheral antenna system (Chls a and b and carotenoids) (Wientjes et al. 2017). In the present study, drought decreased the pigments content (Fig. 1). Drought stress-induced decrease in chlorophyll content has been reported in several plants (Jeyaramraja et al. 2005; Loutfy et al. 2012).

Plants treated with MeJA under both water regimes showed reductions in chlorophyll and carotenoids contents (Fig. 1), which possibly are not related to oxidative stress, since MDA was reduced, indicating greater cell integrity (Fig. 4a). Such results indicate that the degradation of photosynthetic pigments was due to the direct action of MeJA. This response is in agreement with those observed in *Arabidopsis thaliana*, where MeJA application caused a symptom similar to senescence, due to the great decline in photosynthesis and chlorophyll and a strong increase in anthocyanins and activity of antioxidant enzymes (Jung 2004).

The lower content of chlorophylls and carotenoids impairs the use and dissipation of light energy, which can result in reduced photosynthesis (Divya et al. 2018; Lapaz et al. 2019). Therefore, reduction in chlorophyll content may have contributed to the lower A observed in this study (Table 1 and Fig. 1).

Antioxidant pigments and phenolic compounds

Methyl jasmonate application increased anthocyanin content under both water regimes (Fig. 2a), which corroborates results previously found in sweet potatoes (Ghasemzadeh et al. 2016) and *Arabidopsis thaliana* (Jung 2004). In a research carried out by Wang et al. (2013), the accumulation of anthocyanins in leaves, stems, and roots in sweet potato plants was found to play a fundamental antioxidant role in the suppression of reactive oxygen species (ROS) in plants under different abiotic stresses, which corroborates the results of this study (Figs. 2a and 4a), since the application of MeJA reduced MDA (Fig. 4a). Conversely, plants exposed to drought without application of MeJA were still under oxidative stress based on the increase in MDA (Fig. 2a), despite the increase antioxidant pigments and phenolic compounds (Figs. 3 and 4).

Beta-carotene is a nonenzymatic antioxidant produced by a wide range of plant species under stress conditions (Soares et al. 2019), capable of scavenging free radicals that damage cellular organelles (Story et al. 2010). Drought stress and MeJA

application increased the Lβ-car and the Rβ-car (Figs. 2a-b). Similar results were obtained with exogenous application of MeJA in *Moringa oleifera* (Saini et al. 2014). The increase in Lβ-car (Fig. 2a) may be related to a protection mechanism against photodamage in photosystem II (Telfer 2005) mediated by MeJA in sweet potato plants. Kang et al. (2017) verified that transgenic sweet potato plants exhibited increased tolerance to methyl viologen-mediated oxidative stress and resistance to abiotic stressors, such as salt stress, demonstrating that β-carotene plays an essential role in ROS scavenging systems and in protecting the photosynthetic machinery under conditions of oxidative and/or salt stress.

Drought regulates many key genes encoding enzymes of the phenylpropanoid pathway, such as PAL and chalcone synthase, which results in stimulated biosynthesis of phenolic compounds (Sharma et al. 2016). These compounds have antioxidative properties due their capacity to interact with ROS, but also due to their ability to serve as substrate for different peroxidases (Soares et al. 2019), hence plant cells are protected from the negative effects of oxidative stress (Wu et al. 2012). In this context, the PAL enzyme plays a crucial role at the interface between primary and secondary plant metabolism, catalyzing the first step in the biosynthetic pathway of different phenolic compounds (Ghasemzadeh et al. 2016; Sharma et al. 2016). Plants treated with MeJA showed higher PAL activity (Fig. 3e), favoring the production of nonenzymatic antioxidants, such as phenolic compounds, flavonoids, and anthocyanins, especially in plants exposed to drought (Figs. 3a-d), which reflected in lower MDA (Fig. 4a). The effects of drought on the antioxidant system of sweet potato leaves revealed a higher flavonoid content in a tolerant cultivar (Lin et al. 2006).

Proline

Proline is a small neutral amino acid and it is synthesized quickly from the glutamate and/or ornithine pathway in plant cells (Mbinda et al. 2016; Yooyongwech et al. 2013). Proline is able to neutralize, remove and/or transform ROS, allowing the management and sensing of ROS homeostasis and cellular redox balance (Soares et al. 2019). Plants exposed to drought and not treated with MeJA increased proline content in the leaf tissue, however, this effect was potentiated with MeJA application in both water regimes (Fig. 4b) and resulted in lower MDA (Fig. 4a). Previous studies have shown similar results; for example, Anjum et al. (2011) and Mahmood et al. (2012), studying the joint effects of drought and MeJA application on soybeans and bananas, respectively, verified an increase in proline and a reduction in lipid peroxidation in both control and stressed plants.

Partitioned biomass production

Plants exposed to drought reduced partitioned dry biomass production (Tables 3 and 4). This reduction can be explained by the reduction in *A* (Table 1) and possibly due to the reduced mobilization of nutrients caused by the drop in *E* (Table 1). Similar results were found by Yooyongwech et al. (2013) studying 15 sweet potato cultivars.

There was no positive effect of MeJA on dry biomass production, with the exception of ARDB (Table 4). Conversely, MeJA application to cauliflower seedlings significantly increased photosynthesis and chlorophyll content and promoted biomass production under water deficit stress (Wu et al. 2012). In this study, plants treated with MeJA reduced TLA and LDB, but increased ARDB (Tables 3 and 4), suggesting that the assimilated carbon was translocated for the production of adventitious roots, which resulted in less leaf expansion. It may be that, in a less severe drought condition than the one evaluated in this study, the stimulus to form adventitious roots (Table 4) in response to MeJA may be a collaborative mechanism to optimize water absorption. Application of jasmonic acid in pea cuttings increased the formation of adventitious roots in seedlings competent for rooting (Rasmussen et al. 2015). According to Fattorini et al. (2009), jasmonic acid and indolbutyric acid are involved in the success of tobacco rhizogenesis and xylogenesis.

CONCLUSION

Methyl jasmonate application in sweet potato plants affected photosynthetic performance, however it increased the production of antioxidant pigments, phenolic compounds (except Lβ-car under optimum water conditions), and proline.

The evaluated response mechanisms showed that the severity of drought was more prominent than the positive effects of MeJA, since the increases on antioxidant pigments and secondary metabolites were not sufficient to mitigate stress caused by drought, which was reflected in the reduced tuberous root production.

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AUTHOR'S CONTRIBUTION

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SUPPLEMENTARY MATERIAL

Table S1. Summary of analysis of variance (ANOVA) for CO_2 assimilation rate (*A*), stomatal conductance (*gs*), internal concentration of CO_2 in the substomatic chamber (*Ci*), transpiration rate (*E*), instantaneous carboxylation efficiency (*EiC*), water use efficiency (*WUE*), and leaf water potential (Ψw) evaluated on the 3rd, 6th, and 9th day in sweet potato 'Beauregard' treated with two concentrations of a MeJA plant regulator [without (0 µmol·L⁻¹) and with (13 µmol·L⁻¹) application] in combination with two water regimes (normal and drought conditions, which correspond to field capacity at 100 and 40%, respectively).

Stress days	Source of variation	*A*	*gs*	*Ci*	*E*	*EiC*	*WUE*	*Ψw*
3rd	Water regimes	***	***	***	***	***	**	*
	MeJA	*	ns	***	ns	**	ns	ns
	Interaction	ns	ns	ns	ns	*	ns	ns
6th	Water regimes	***	***	***	***	***	***	***
	MeJA	***	ns	**	***	***	*	ns
	Interaction	ns	ns	ns	ns	*	*	ns
9th	Water regimes	***	***	***	***	***	*	*
	MeJA	***	ns	***	**	***	*	ns
	Interaction	ns	ns	ns	ns	ns	ns	ns

Significance by factorial analysis: ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table S2. Summary of analysis of variance (ANOVA) for chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Tchl), carotenoids (CAR), total anthocyanin (TA), leaf β-carotene (Lβ-car), and root β-carotene (Rβ-car) evaluated on the 9th day in sweet potato 'Beauregard' treated with two concentrations of a MeJA plant regulator [without (0 µmol·L⁻¹) and with (13 µmol·L⁻¹) application] in combination with two water regimes (normal and drought conditions, which correspond to field capacity at 100 and 40%, respectively).

Source of variation	Chl *a*	Chl *b*	Tchl	CAR	TA	Lβ-car	Rβ-car
Water regimes	***	***	***	***	***	***	***
MeJA	***	***	***	***	***	ns	***
Interaction	***	***	***	***	***	***	***

Significance by factorial analysis: ns, not significant; ***, $p < 0.001$.

Table S3. Summary of analysis of variance (ANOVA) for total phenolic in leaf (TPL) and root (TPR), total flavonoid in leaf (TFL) and root (TFR) activity of the enzyme phenylalanine ammonia lyase (PAL), malondialdehyde (MDA), and proline evaluated on the 9th day in sweet potato 'Beauregard' treated with two concentrations of a MeJA plant regulator [without (0 µmol·L⁻¹) and with (13 µmol·L⁻¹) application] in combination with two water regimes (normal and drought conditions, which correspond to field capacity at 100 and 40%, respectively).

Source of variation	TPL	TPR	TFL	TFR	PAL	MDA	Proline
Water regimes	***	***	***	***	***	***	***
MeJA	***	***	***	***	***	***	***
Interaction	***	***	***	***	***	***	***

Significance by factorial analysis: ***, $p < 0.001$.

Table S4. Summary of analysis of variance (ANOVA) for total leaf area (TLA), number of tuberous root (NTU), diameter of tuberous root (DTU), leaf (LDB), steam (SDB) total shoot (TSDB), tuberous root (TUDB), adventitious root (ARDB), and total root dry biomass (TRDB) evaluated on the 9th day in sweet potato 'Beauregard' treated with two concentrations of a MeJA plant regulator [without (0 µmol·L⁻¹) and with (13 µmol·L⁻¹) application] in combination with two water regimes (normal and drought conditions, which correspond to field capacity at 100 and 40%, respectively).

Source of variation	TLA	NTU	DTU	LDB	SDB	TSDB	TUDB	ARDB	TRDB
Water regimes	***	*	***	***	***	***	***	ns	***
MeJA	*	ns	ns	**	ns	ns	ns	**	ns
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns

Significance by factorial analysis: ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.