Research paper

Drought and heatwave affected the African eggplant differently when present in combination than individually

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ABSTRACT

Heat waves and drought periods are set to happen more often due to a changing climate, especially in Africa. The African eggplant, *Solanum aethiopicum* L., is a highly nutritious vegetable crop indigenous to Africa commonly grown by small-holder farmers. It is adapted to harsh environments but empirical data on its tolerance to high temperatures and drought individually or in combination to help farmers is lacking. This project aimed to describe the effects of drought in combination with heat waves on the leafy African eggplant Shum E11 genotype over 26 days of stress. Four-week-old plants were subjected to repetitive heatwave-type stress consisting of eight days of day/night temperatures reaching 32/26 °C or 37/31 °C compared to 27/21 °C, followed by four days of recovery, repeated twice. A drought treatment was applied during the experiment by maintaining the soil water potential at − 100 kPa compared with − 10 kPa for non-stressed plants. Drought stress had a predominant effect over heat by reducing leaf production and leaf membrane stability and increasing chlorophyll content. Antioxidants and phenols were unaffected by drought or heat stress while mineral elements were impacted differently by each stress and their combination. These results indicate a good tolerance of the E11 genotype to heat stress during the vegetative stage while drought negatively affected plant growth and leaf biochemical compounds. Further research on later developmental stages will complete our understanding of this variety's tolerance to heat and drought and its potential role in fields affected by environmental stresses.

1. Introduction

Climate change increases the likelihood of extreme events such as drought and heatwaves worldwide (Mbow et al., 2019). Both of these stresses already significantly limit crop production, especially in Africa (Niang et al., 2014). Drought stress is a consequence of insufficient rainfall or deficit soil moisture and can lead to various morphological, physiological or biochemical defects in plants (Farooq et al., 2009). Heatwaves, and heat stress in general, also have deleterious effects on plant growth especially at the reproductive stages (Zhou et al., 2022; Bita and Gerats, 2013). These stresses are often associated in the field, making it necessary to study their combination on plant responses (Lamaoui et al., 2018). Most studies have, however, focused on the responses of various crops to individual stresses to uncover the mechanisms of stress tolerance and sensitivity (Suzuki et al., 2014; Fahad et al., 2017).

A combination of stresses can lead to different, and often more

severe, responses than individual ones such as seen in wheat (Yousaf et al., 2022) or citrus plants (Zandalinas et al., 2016). Despite some shared responses between plants under stress, signaling pathways induced by combined stress can sometimes be different or opposite to the ones induced by individual stresses (Suzuki et al., 2014). Chlorophyll, the primary photosynthetic pigment, is, for example, degraded under drought due to the instability of the chlorophyll membrane, increased photo-oxidation, and decreased chlorophyll production (Parkash and Singh, 2020). Under high temperatures, the photo-synthetic apparatus might be under-performing due to reduced enzymatic activity (Hassan et al., 2021). The combination of heat and drought might thus reduce photosynthetic activity on multiple fronts, making one cultivar tolerant to one stress not adapted to the stress combination (Fahad et al., 2017). Stress also increases the production of reactive oxygen species which can damage proteins, cells, and DNA when present at high levels and need detoxification by antioxidants (Fahad et al., 2017). The variety of responses observed in crops, such as an increase in

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total phenols and a decrease in anthocyanins under high heat and an increase in antioxidant enzymes under drought, might lead to new pathways activated under their combination (Fahad et al., 2017).

To increase field resilience to environmental stress, the use of indigenous crops has recently attracted attention due to their higher tolerance to stress. The African eggplant (*Solanum aethiopicum* L.) Shum is an indigenous vegetable from Africa and a close relative to the common brinjal eggplant (*S. melongena* L.) and tomato (*S. lycopersicum* L.) (Aubriot and Daunay, 2019). It is mostly grown by small-holder farmers in peri-urban areas for its edible leaves (Schippers et al., 2000). The leaves are rich in nutrients but their market value can be highly affected by limited water supply as leaf quality can deteriorate quickly (Han et al., 2021). Every Shum accession studied by Nakanwagi et al. (2017) had a decrease in leaf and stem growth under drought, however, a high variation was observed with some cultivars recovering much better than others, indicating some genetic basis of tolerance. Drought also affected the secondary metabolism of *S. aethiopicum* such as carotenoids and sugars (Mibei et al., 2017; Mibei et al., 2018). The heat tolerance of *S. aethiopicum*, in particular Shum varieties, has not been investigated to our knowledge.

The main objective of this study was to clarify the effects of drought and repetitive heat stress, and their combination, on a *S. aethiopicum* Shum genotype in terms of leaf and stem development, chlorophyll levels, leaf nutrient concentrations, proteins, carbohydrates, and non-enzymatic antioxidant content. It was hypothesised that the combination of drought and heat stress would cause specific responses that differ from individual stress responses. This study will help understand how the African eggplant responds under stress, necessary to use this crop to its potential in changing climates.

2. Materials and methods

2.1. Plant material and growth conditions

A pot experiment was carried out to investigate the responses of the African eggplant Shum (*Solanum aethiopicum* L. genotype E11) to a combination of heat and drought stress. The Uganda Christian University generously provided us with the seeds. Before use, seeds were disinfected by immersion in 10% trisodium phosphate decahydrate solution for three hours. They were then washed with distilled water, placed for 1 h in a dilution of 70% water:30% commercial bleach, and rewashed. Seeds were then left to dry at room temperature for 15 days. At the start of the experiment, seeds were placed in 7 cm × 7 cm germination pots filled with John Innes No.2 compost (2 loam:1 peat:1 sand) (Westland®, J Arthur Bower's). Pots were placed in an MLR-352 growth cabinet (PHC Holdings Corporation®, Tokyo, Japan) set at 70% relative humidity and 27/21 °C for a 12 h photoperiod, and watered from the bottom when needed. Thirty seedlings were transplanted in 3 L pots after four weeks and placed following a split-plot design in three growth cabinets (Plant growth chamber A1000, Conviron®, Winnipeg, Canada). Valoya LEDs lights (BX NSI spectrum 'white' LED, Valoya®, Helsinki, Finland) providing 350 μmol m^{-2} s^{-1} light intensity were fitted in the cabinets. Natural predators were applied once, four days after transplanting (Anderline, Starskii, Chrysoline C, Bioline AgroSciences®, Little Clacton, UK). The final harvest of plants took place 36 days after transplanting.

2.2. Treatment application

Plants were left to acclimate for seven days in the growth cabinets and were well-watered. Irrigation was withheld for five plants in each cabinet for four days until the soil matric water potential (SWP) reached − 100 kPa as monitored by MPS-2 sensors (Decagon devices, Meter Environment®, Pullman, WA, United States) connected to a datalogger EM50g (Meter Environment®) and maintained at this level after that. This water stress level was selected based on previous research (data not shown) and on the relationship between SWP and water content in which case − 100 kPa represents roughly a 50% decrease in water content. This level is often used in drought experiments to trigger water stress in plants (Evett, 2007). The other 15 plants (five in each cabinet) were irrigated to maintain an SWP of approximately − 10 kPa. Soil water content was also monitored daily using an HH2 moisture meter attached to a WET sensor (Delta-T devices®, Burwell, UK). When plants reached the required SWP under deficit irrigation, the heat treatment was initiated in a gradual way to reduce the risk of an osmotic shock and mimic natural conditions. Two sequential heatwave-type stresses were imposed with a maximum of 32/26 °C (T32) and 37/31 °C (T37) day/night temperature compared to 27/21 °C (T27) for control plants. The heat increased gradually over day one (1 DAHS, days after heat stress) and was maintained for seven days, followed by a six-day recovery period at T27. The temperature rose again after the recovery period (14 DAHS) over eight days, followed by a second recovery period. This level of heat was selected based on temperatures sometimes reached in Central Africa, where Shum genotypes are mostly grown, during heat waves with T37 being on the higher end of the average temperature in Central African countries under normal conditions (Worlddata, 2023). The daily schedule for each of the treatments for one heat-wave period is shown in (Fig. 1).

2.3. Growth and physiological measurements

2.3.1. Leaf expansion rate

Two young leaves (less than 30 mm in length) were labelled on each plant at a similar height and developmental stage at 0 DAHS. Their length and maximum width were recorded three times a week. The growth difference between the measurements was considered to be the daily length and width expansion rates, respectively. On the day before the second heatwave (13 DAHS), new young leaves were labelled to limit the impact of ageing on the results.

2.3.2. SPAD index

The SPAD index, measuring the transmittance of red and infrared through the leaf, was measured three times a week at midday from 1 to 26 DAHS using a MultispeQ V2.0 device with Photosynthesis RIDES 2.0 protocol (PhotosynQ®, East Lansing, MI, United States) (Kuhlgert et al., 2016).

2.3.3. Destructive harvest

The leaf area and weight of a sub-sample representing a mix of developing and developed leaves were measured on the last day of the experiment using a two-digit balance and a Li-3000 area meter (LiCor®, Lincoln, NE, United States). This sub-sample was then stored at − 80 °C for biochemical analysis. The rest of the leaves were weighed after measuring their total leaf area. Stem weight was recorded before the shoot biomass was oven-dried at 80 °C until reaching a constant weight.
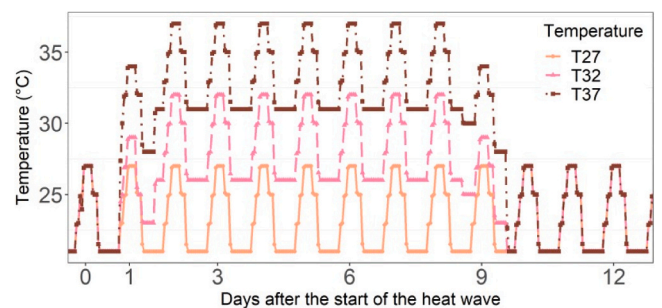

Fig. 1. Daily temperature schedule of 27 °C (T27), 32 °C (T32), and 37 °C (T37) treatments throughout one heatwave. This treatment was repeated twice between 1 and 26 days after the start of the heat waves.

2.3.4. Leaf electrolyte leakage

At the end of the second recovery period (26 DAHS), one leaf disc (10 mm diameter) was sampled from five fully expanded leaves. Each disc was washed three times with distilled water before being placed in 20 mL of distilled water. After incubating the discs at room temperature on a shaker (200 rpm) for 20 h, the electrical conductivity of the bathing solution (EC1) was recorded (LAQUAtwin EC-33 meter, Horiba®, Kyoto, Japan). A second electrical conductivity measurement (EC2) was taken after incubating the discs in a water bath at 95 °C for 60 min. The electrolyte leakage (EL) was expressed as % EL and calculated as follows:

$$\%EL = EC1/EC2 * 100 \tag{1}$$

2.4. Biochemical analysis

2.4.1. Leaf chlorophyll content

Wintermans and De Mots (1965) protocol was followed to determine leaf chlorophyll content on the last day of the experiment. Two leaf discs (15 mm diameter) were sampled from the first two fully expanded leaves and placed in 10 mL 95% cold ethanol (Fischer Scientific, Hampton, NH, United States). After 48 h in the dark at 4 °C, the absorbance of the supernatant was read at 470, 649, and 665 nm (Ultrospec III, Pharmacia LKB, Stockholm, Sweden). The amount of chlorophyll a (Chl a), b (Chl b), and carotenoids (Car) per unit area was calculated as follows:

$$Chl\ a = (13{\cdot}95 * A649)/1{\cdot}54 \tag{2}$$

$$Chl\ b = (24{\cdot}96 * A649 - 7{\cdot}32 * A665)/1{\cdot}54 \tag{3}$$

$$Car = (10^3 * A470 - 2{\cdot}05 * Chl\ a - 114{\cdot}8 * Chl\ b)/1{\cdot}54 \tag{4}$$

2.4.2. Total carbohydrates

Total carbohydrates were quantified following the phenol-sulfuric acid colourimetric method using glucose (Merck, Darmstadt, Germany) as a standard (Dubois et al., 1956). Fresh leaf tissue (300 mg) from the sub-sample obtained on the last day of the experiment was digested in 10 mL of 80% cold methanol (Fischer Scientific) and 100 μL of the digested sample was added to 900 μL of distilled water, 2.5 mL sulfuric acid (Fischer Scientific), and 500 μL of a 5% phenol (Fischer Scientific) solution. After 20 mins at room temperature, the absorbance of the solution was read at 490 nm using a UV–VIS spectrophotometer (Ultrospec III).

2.4.3. Total soluble protein

The procedure of Bradford (1976) was followed to quantify total proteins on the last day of the experiment. Briefly, 50 mg of dry leaf sample was digested in 3 mL of 100 mM sodium phosphate buffer (pH 7.0 at 20 °C) (Fischer Scientific) and 10 μL of the supernatant was pipetted in a 96- well plate (96-Well Microtiter™Microplates, Fischer Scientific). Then, 290 μL Bradford reagent (Merck) was added to each well. The plate was incubated on a shaker for 5 mins at 200 rpm at room temperature. The sample absorbance at 595 nm was measured using a plate reader (CLARIOstar, BMG Labtech, Ortenberg, Germany) and bovine serum albumin (Merck) was used as a standard.

2.4.4. Total antioxidants

The Trolox Equivalent Antioxidant Capacity (TEAC) method was used to determine total antioxidants of the plants at the end of the stress period with trolox (Merck) used as a standard (Re et al., 1999). A solution of 7 mM ABTS (Merck) and 2.45 mM potassium persulfate (Merck) was made and left in the dark overnight. When ready to use, this solution was diluted with ethanol (Fischer Scientific) until it reached an absorbance of 0.9 at 734 nm and warmed on a hotplate at 40 °C.

Approximately 50 mg of dry leaf sample was digested in 5 mL of 80% cold methanol (Fischer Scientific) and 30 μL of the sample supernatant was pipetted in 3 mL of the diluted ABTS working solution and placed in a water bath at 40 °C for 15 min. After the time elapsed, the absorbance of the samples was read at 734 nm in a UV–VIS spectrophotometer (Ultrospec III).

2.4.5. Total phenols

The Folin-Ciocalteau method described by Singleton, Orthofer, and Lamuela-Raventòs (1999) was used to determine leaf phenols using gallic acid as a standard. A fresh leaf sample (300 mg) from the plants on the last day of the experiment was digested in 10 mL of 80% cold methanol (Fischer Scientific) and 300 μL of the digested sample was added to 600 μL of 10% Folin-Ciocalteau reagent (Merck). After 2 mins, 2.5 mL of 700 mM sodium carbonate (Fischer Scientific) was added. The mixture was left to stand at room temperature for 1 h, after which the absorbance of each sample was read at 765 nm using a UV–VIS spectrophotometer (Ultrospec III).

2.4.6. Leaf nutrient concentration

Three hundred milligrams of dried and milled leaf sample from the last day of the experiment was digested in 6 mL of nitric acid and placed in a digestion microwave (Multiwave PRO, Anton Paar, Graz, Austria) reaching 175 °C over a 20 mins period and maintained for a further 20 mins followed by a 10 mins cool down at 55 °C. The digested sample was diluted using 18.2 milli-Q water for analysis by ICP-MS (Inductively Couple Plasma Mass Spectrophotometry, Thermo- Fisher Scientific iCAP-Q; Thermo Fisher Scientific, Germany). A multi-element solution (Claritas- PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, United States) and a bespoke external multi-element calibration solution (PlasmaCAL, SCP Science, Quebec, Canada) were used as calibration standards.

Leaf nitrogen concentration was analysed separately using a FlashEA® 1112 elemental analyser (Thermo Fischer Scientific, Waltham, MA, United States). A dried and milled sample (50 mg) was placed in a foil capsule. Elemental nitrogen was detected after the conversion and filtration of the gas mixture generated by combustion, providing a nitrogen percentage.

2.5. Statistical analysis

Statistical studies were performed on R version 4.1.2. The data were assessed for normality and variance homogeneity of residuals. Normality was checked using a combination of the Shapiro test and visual evaluation (density plot, Q-Q plot, and histogram). Variance homogeneity was assessed using Levene's test and visual inspection of residuals. A two-way analysis of variance (ANOVA) test was then performed with "Irrigation" and "Temperature" as the main factors and including their interaction. For time series, a repetitive measure model was fitted (lmer) with two random effects including the repetitive measures on each plant and the interaction between the individual plants and the measurement dates. Tukey test was used to perform pairwise comparisons among both irrigation and temperature groups at a 0.05 significance level.

The p values of each factor are shown alongside the figures of the measured variables. No p values are displayed for time series for clarity. In each figure, stars denote the significance level of the pairwise comparisons by the Tukey test when the significant factor has more than two levels (i.e. Temperature) while no pairwise comparisons were done on the two-level factor (i.e. Irrigation). When the interaction of the factors was significant, letters were used to display the significance for clarity.

3. Results

3.1. Plant development and characteristics

The effects of the stress throughout time are shown in Fig. 2. Water stress reduced the length and width leaf expansion rate (LER) at 3 and 5 DAHS compared to the control (Fig. 3). Only length LER was further slowed on day 7, while width LER was maintained at each irrigation level. After that,

water stress did not significantly impact either LER (Fig. 3). T32 increased LER from 5 to 13 DAHS, at the end of the first heatwave (Fig. 3). During the recovery period, however, the LER of heat-stressed plants slowed down quickly and reached the same level as T27 plants (Fig. 3). LER continued to decrease throughout the recovery, with every temperature treatment following the same trend (Fig. 3). The newly labelled leaves during the second heatwave had a noticeably slower growth for every treatment, peaking at the end of the heatwave (Fig. 3). The decrease in daily LER noted for every treatment during the first recovery period was not present during the second heatwave with plants maintaining their LER from the last heatwave day (Fig. 3).

Despite the maintenance of LER under limited irrigation for most of the treatment period, the final leaf dry weight and total leaf area were significantly reduced for plants under water stress (Fig. 4a,c). Heat stress, in contrast, did not impact leaf dry weight (Fig. 4a). Under full irrigation, plants grown at T32 had a larger leaf area than the ones grown at T27 but this was not seen under deficit irrigation (Fig. 4c). Stem dry weight was reduced under deficit irrigation (Fig. 4b). T32 increased stem dry weight compared to T27 only under full irrigation (Fig. 4b). Electrolyte leakage increased significantly under drought (Fig. 4d). Under deficit irrigation, EL was reduced by T37 compared to T27 (Fig. 4d).

3.2. Leaf chlorophyll content

The SPAD index values were consistently and significantly increased by deficit irrigation from 3 DAHS (Fig. 5). During the heatwave, the highest temperature increased SPAD values only for plants under water stress (Fig. 5). During the first recovery, however, the SPAD of heat-stressed plants quickly dropped while T27 plants maintained their levels (Fig. 5). At the beginning of the second heatwave, there was a rapid increase in chlorophyll levels among T37 plants, especially those subjected to both the heat and water stress (Fig. 5). This increase was temporary as the levels reverted back to those of T27 plant levels by 20 DAHS. During the initial heatwave, T32 SPAD levels exhibited a slight increase, aligning with the pattern observed in T27 plants. However, in the subsequent heatwave, when subjected to full irrigation, these levels declined and only recovered during the recovery period (Fig. 5). On the last day of the experiment, only the irrigation treatment had a significant effect, marked by an increase for plants under water stress (Fig. 5).

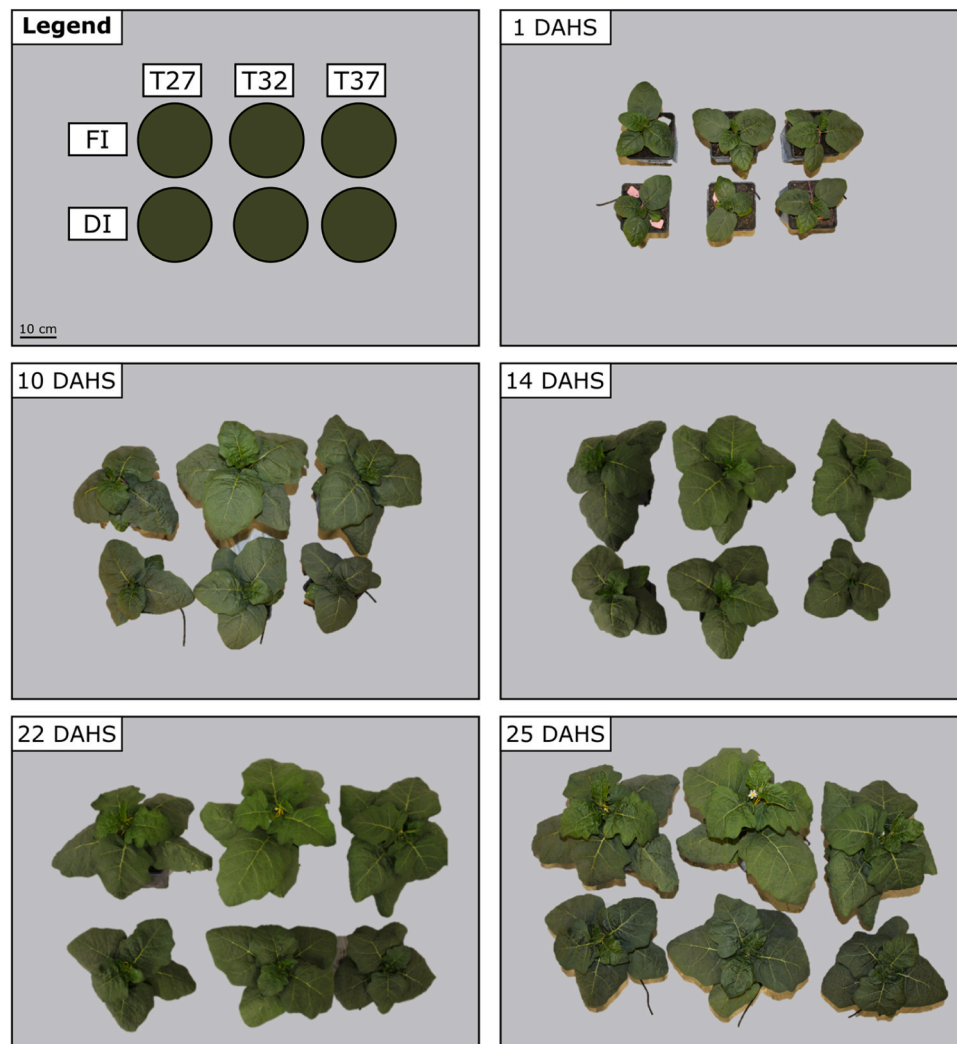At the end of the experiment, chlorophylls a, b, and carotenoids were

Fig. 2. Pictures of the plants at 1, 10, 14, 22, and 25 days after the start of heat stress (DAHS). FI = Full irrigation, DI = Deficit irrigation.
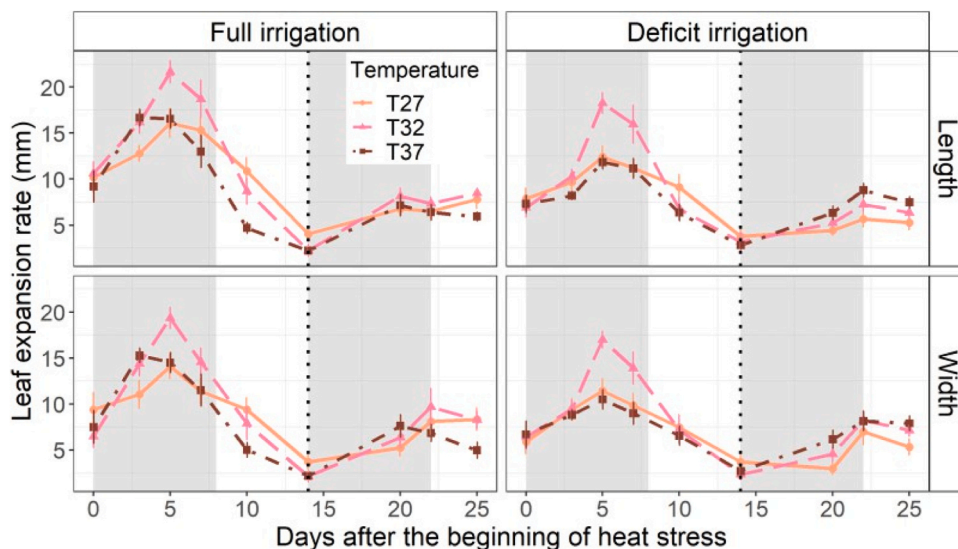
Fig. 3. Leaf length (top row) and width (bottom row) expansion rate throughout the stress period. The greyed areas delimit the heat wave periods. The dotted vertical line highlights the newly labelled leaves.
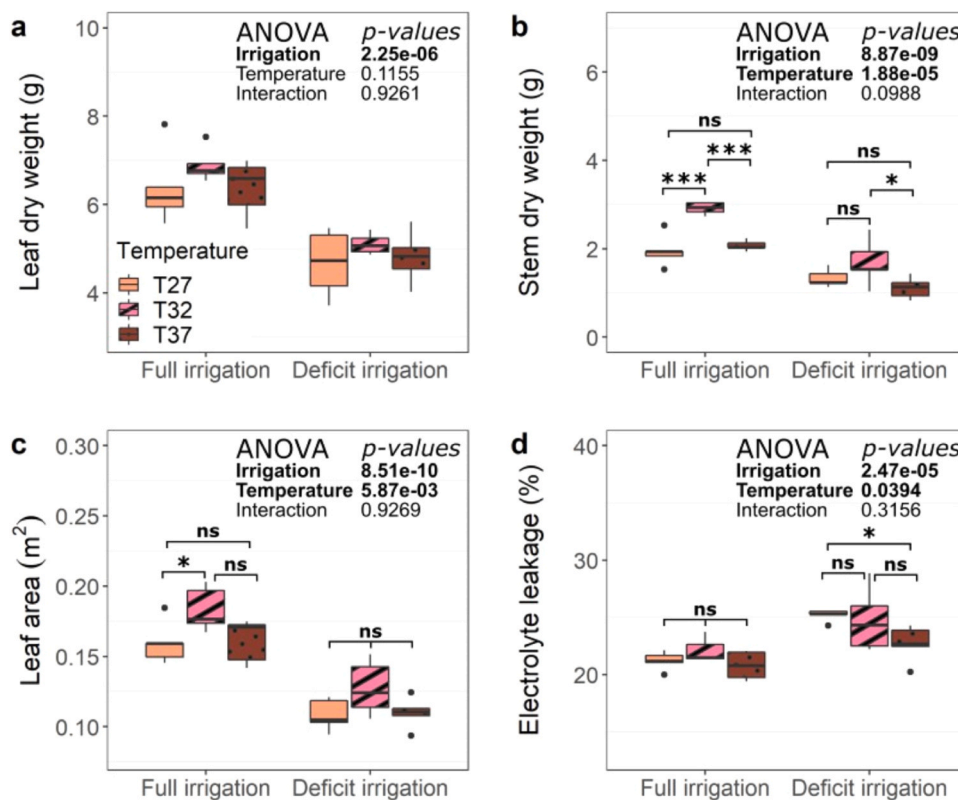

Fig. 4. Plant morphological characteristics of the African eggplant at the end of the 26 days under increased temperature and/or reduced irrigation treatment. Sub-plots represent individual plant's (a) leaf dry weight, (b) stem dry weight, (c) leaf area, and (d) electrolyte leakage. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 5). Black dots represent outliers. Stars denote the significance level of the pairwise comparisons by the Tukey test (* p < .05, ** p < .01, and *** p < .001.). NS = Non Significant.

increased under deficit irrigation (Fig. 6a,b,c). The ratio of chlorophyll a over chlorophyll b was not affected under either stress or their combination (Fig. 6d).

3.3. Biochemical analysis

No differences were noted in leaf antioxidant levels between temperature treatments under full irrigation but an increase in T32 plants when compared to T37 was noted under deficit irrigation (Fig. 7a). Neither deficit irrigation nor increasing temperature impacted the levels of leaf phenols (Fig. 7b). Under full irrigation, the protein concentration was reduced for T32 plants when compared to T27 plants, which had the highest level (Fig. 7c). Under deficit irrigation, however, T27 and T32 had a similar concentration of protein while T37 displayed
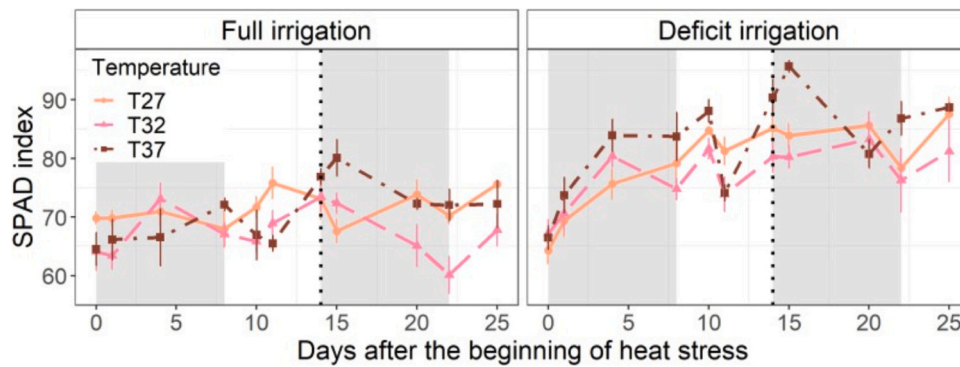
Fig. 5. Chlorophyll index (SPAD) throughout the stress period. The graph on the left represents the full irrigation treatment and deficit irrigation is on the right. The greyed areas represent the heat wave periods.
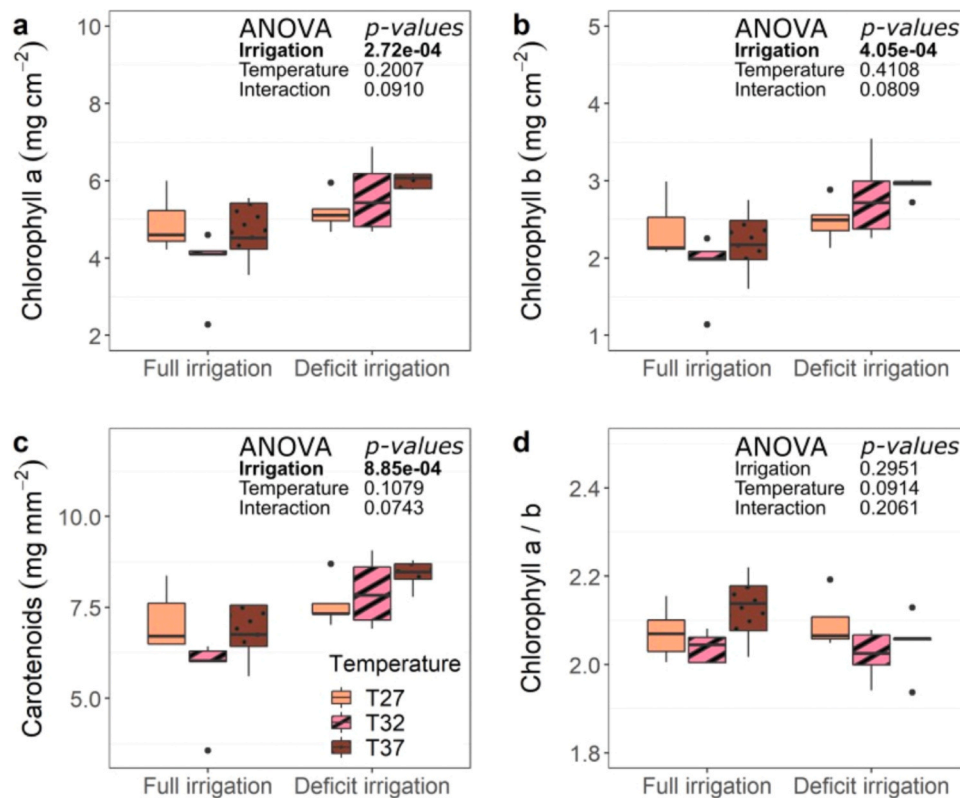

Fig. 6. Leaf pigment content per unit area of the African eggplant after 26 days under increased temperature and/or reduced irrigation treatment. Sub-plots represent (a) chlorophyll a, (b) chlorophyll b, (c) carotenoids, and (d) the ration of chlorophyll a over chlorophyll b. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 5). Black dots represent outliers. The p-values are displayed in bold when significant alongside the statistical test used.

an increased level (Fig. 7c). Irrigation only reduced leaf protein levels at T27 (Fig. 7c). Total carbohydrates increased linearly with heat for plants well irrigated but were only significantly higher for plants grown at T37 when compared to T27 (Fig. 7d). Carbohydrates were not significantly impacted by the different temperatures when plants were under deficit irrigation and the irrigation treatment did not impact their levels either (Fig. 7d).

3.4. Leaf nutrient concentration

Leaf phosphorus concentration decreased under drought and increased at the highest temperature level (Table 1). Potassium leaf concentration was not significantly affected by either drought, heat, or their interaction (Table 1). While leaf magnesium and calcium concentrations were not significantly different between heat treatments, deficit irrigation decreased both nutrients (Table 1). Leaf boron concentration was significantly reduced under deficit irrigation across all temperature levels and the highest heat treatment resulted in a positive effect on leaf boron concentration (Table 1). Leaf sodium concentration was reduced under deficit irrigation and unaffected by the different temperatures (Table 1). The T37 treatment increased leaf manganese levels when compared to T27 and T32 plants under both irrigation levels (Table 1). The stress interaction caused a reduction in leaf iron concentration for T32 plants under full irrigation and an increase in plants at T37 under deficit irrigation (Table 1). Reduced irrigation increased leaf zinc and selenium concentrations at every temperature while their concentration remained unaffected by the temperature treatment (Table 1).
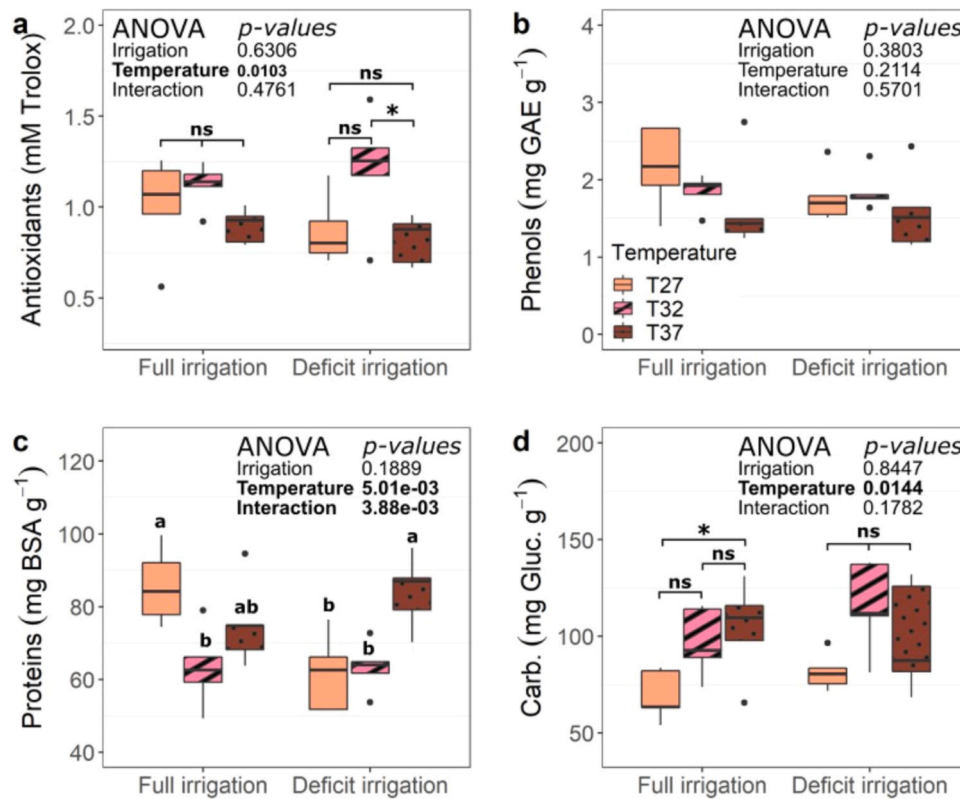
Fig. 7. Leaf biochemical analysis of the African eggplant at the end of the 26 days under increased temperature and/or reduced irrigation treatment. Sub-plots represent (a) total antioxidants, (b) total phenols in mg gallic acid equivalent (GAE) per g fresh weight, (c) total proteins in mg bovine serum albumin (BSA) per g dry weight, and (d) total carbohydrates in mg glucose (Gluc) per g fresh weight. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 5). Black dots represent outliers. The p-values are displayed in bold when significant alongside the statistical test used. Stars denote the significance level of the pairwise comparisons by the Tukey test at 0.05% level of significance with * for p < .05, For measures where the interaction of the parameters was significant after an ANOVA, letters are used to display the difference between treatments for clarity. Boxplots not sharing any letters are significantly different in this case. NS = Non Significant.

Table 1

Analysis of variance and mean comparisons for leaf nutrient concentrations of African eggplants grown under different irrigation and temperature levels'.

Source of variance	P	K	Mg	Ca	B	Na	Mn	Fe	Zn	Se
	g kg^{-1}				**mg kg^{-1}**					**µg g^{-1}**
Irrigation (I)	*	ns	* **	* *	* **	*	* **	* **	* **	*
Temperature (T)	*	ns	ns	ns	* *	ns	* **	* *	ns	ns
I x T	ns	ns	ns	ns	ns	ns	*	* *	ns	ns
Irrigation										
Full irrigation	4.44[a]	37.87	6.59[a]	30.27[a]	40.83[a]	56.79[a]	118.0	80.64	14.40[b]	8.11[b]
Deficit irrigation	3.80[b]	39.41	5.49[b]	24.65[b]	23.59[b]	37.26[b]	97.47	108.9	19.56[a]	9.16[a]
Temperature										
T27	3.81[b]	39.43	6.49	28.30	28.75[b]	38.14	73.76	96.89	16.02	8.54
T32	3.76[b]	36.94	5.73	26.89	31.75[ab]	45.17	90.93	85.76	16.51	9.02
T37	4.78[a]	39.56	5.90	27.20	36.13[a]	57.76	158.5	101.7	18.62	8.35
I x T										
Full irrigation + T27	4.38	41.05	6.97	28.61	37.72	36.79	77.15[c]	93.14[bc]	14.03	8.31
Full irrigation + T32	3.92	34.68	6.33	31.00	39.21	58.06	97.04[c]	68.78[d]	13.76	8.57
Full irrigation + T37	5.02	37.87	6.48	31.20	45.56	75.51	179.9[a]	79.99[cd]	15.28	7.45
Deficit irrigation + T27	3.25	37.81	6.02	27.99	19.77	39.49	70.36[c]	100.6[b]	18.01	8.77
Deficit irrigation + T32	3.59	39.19	5.12	22.77	24.30	32.38	84.83[c]	102.7[b]	18.71	9.48
Deficit irrigation + T37	4.55	41.24	5.33	23.20	26.71	40.02	137.2[b]	123.3[a]	21.95	9.24

P = Phosphorus, K = Potassium, Mg = Magnesium, Ca = Calcium, B = Boron, Na = Sodium, Mn = Manganese, Fe = Iron, Zn = Zinc, Se = Selenium. NS, * , * *, * ** Non-significant or significant at P ≤ 0.05, 0.01, 0.001, respectively. Means not sharing any letters within each column are statistically different according to Tukey's test (P = 0.05).

4. Discussion

While a few groups have researched the effects of drought on the African eggplant, the effects of drought in combination with heat have not been studied despite the combination of these stresses commonly observed in the field (Suzuki et al., 2014; Sseremba et al., 2018; Nakanwagi et al., 2020). In some cases, the stress combination does not increase plant damage further than the single stresses when one of the stresses is predominant, as was the case in this study for leaf development and chlorophyll content, and was previously observed in tomato

(Zhou et al., 2017; Nankishore and Farrell, 2016). For other measures, the stress combination can lead to new responses as reported by Duan et al. (2017) and observed in this current study for leaf protein and some mineral elements. Deficit irrigation had previously been reported to reduce leaf area, number, and dry weight in the African eggplant as observed in this current study (Limbu, Sharma, and Rao, 2018; Nakanwagi et al., 2017; Gaveh et al., 2011). Reducing leaf area is part of avoidance mechanisms aiming at reducing water loss through evapotranspiration (De Micco and Aronne, 2012). Heat stress can, in contrast, promote leaf production as seen in lettuce (*Lactuca sativa* L.) and amaranth (*Amaranthus tricolor* L.) due to improved carbon assimilation leading to improved growth, and observed in this study at T32 (Sublett, Barickman, and Sams, 2018; Hwang, Chao, and Lin, 2018). The increase in leaf area was not noted at the highest temperatures tested, suggesting that larger leaves are not beneficial under temperatures too high as they reduce the plant's ability to regulate leaf temperature and sustain cellular processes (Hassan et al., 2021).

Moreover, the increase in leaf area at T32 was not noted under deficit irrigation, showing the predominant effect of drought in this case and the limited beneficial effects of increased temperatures. The non-negative effects of these temperatures on the African eggplant growth led to the absence of further damage caused by the combination of stresses, in contrast to previous observations in chickpea and barley, showing that the stress combination was not antagonistic in this case (Awasthi et al., 2014; Rollins et al., 2013). The reduced growth is often due to damage to the cell membranes and other compounds by reactive oxygen species produced under stress. Reactive oxygen species formation under stress is balanced by the presence of antioxidant compounds which are essential for their removal (Fahad et al., 2017). Total antioxidants and phenols were not increased under stress in this current study. A study by Hwang, Chao, and Lin (2018) showed no differences in antioxidant capacity under drought in amaranth while only one tolerant tomato cultivar had an increase in phenols under heat in a study by Zhou et al. (2015). Independently of the drought tolerance of the cultivars used, an increase in leaf and fruit antioxidant compounds was, however, previously noted in tomato, eggplant, and spinach under heat (Klunklin and Savage, 2017; Plazas et al., 2022; Khosa et al., 2022). Under the combination of heat and drought, enzymatic antioxidants were either reduced or unchanged in tomato after long-term stress, supporting results observed in this current study (Zhou et al., 2019). These observations highlight the variability of responses to detoxify cells depending on growth stage, stress intensity, and plant organ.

Another important stress tolerance mechanism is the increase in soluble sugars due to their role in leaf turgor maintenance (Lamaoui et al., 2018). Total carbohydrates were increased linearly under heat stress in this current study as reported previously in potato (Naz et al., 2018). Sugar levels were increased under mild water stress in tomato but decreased under a more severe stress, showing a dynamic response to the stress intensity (Sun et al., 2016). Stress combination affected sugar levels differently in the study by Zhou et al. (2017), highlighting an interplay of heat and drought in carbohydrate metabolism as seen in this current study with an increase driven by heat only under full irrigation. Indeed, despite drought alone not impacting carbohydrate levels in the current study, the presence of this stress impacted negatively the increase noted at T37. This might be due to the effect of drought on photosynthesis, whose products is sugars. While under full irrigation photosynthesis is not limited and sugars can accumulate to offer heat protection, deficit irrigation might reduce the photosynthesis potential needed for an increase under heat without significantly reducing the baseline sugar production (Zhou et al., 2017).

Under heat alone, an increase in SPAD, directly proportional to the absolute chlorophyll content (Jiang et al., 2017), was observed in tomato (Ro et al., 2021; Bhattarai et al., 2021). This was in line with the observations made in this current study during the heatwave period, especially for plants under deficit irrigation. This can be an acclimation response to heat where plants promote chlorophyll production and/or

thermostability to compensate for the chlorophyll degradation pathway initially triggered under heat (Wang et al., 2018; Havaux, 1993). Chlorophyll content might also have increased per unit area due to smaller leaves being produced, without an increase in production or stability. However, it has to be noted that SPAD measurements can be affected by leaf water status, which was affected in this current study and might have thus interfered with the accurate chlorophyll content (Martìnez and Guiamet, 2004; Schlemmer et al., 2005). The final chlorophyll content, measured following a destructive method, was unaffected by heat. A recovery period after heat stress in tomato also led to the absence of effect at the end of the experiment despite an increase during the heat stress period (Lee et al., 2023). This may be due to a fast response of chlorophylls and carotenoids to outside temperatures to limit photodamage under lower temperatures. Chlorophylls a and b and carotenoids were, however, still increased by the drought stress, as observed throughout the stress with the SPAD measurements. Despite an increase under drought alone, chlorophylls a and b in *S. chilense* and *S. lycopersicum* decreased under the combination of heat and drought, following the same trend as under heat alone and highlighting the predominant effect of heat, in contrast to observations made in this current study (Blanchard-Gros et al., 2021). The predominance of one of the stresses might be related to the adaptation of the crop of interest with drought being predominant over heat stress in this current study due to the long-term adaptation of the African eggplant to high temperatures.

Electrolyte leakage has been used routinely as an indicator of membrane damage and is a useful parameter to identify stress-tolerant genotypes (Bajji, Kinet, and Lutts, 2002; Alsadon, Wahb-Allah, and Khalil, 2006; Aleem et al., 2020). While the increase observed in EL under deficit irrigation in the current study is consistent with previous observations in eggplant and tomato, the reduction of EL following moderate heat stress and recovery under deficit irrigation has not been reported previously (Cornejo-Rìos et al., 2021; Kirnak et al., 2001, 2002). The highest temperature might have played a protective role against cell membrane damage caused by drought through other mechanisms in place above a certain temperature. A lower EL was associated with beneficial traits under heat such as maintained pod set or grain yield in cowpea (Thiaw and Hall, 2004). After a recovery period, EL was quick to recover in wheat and tomato despite a marked increase during the stress period, hinting at a very dynamic process (Grigorova et al., 2011; Lee et al., 2023). The decrease of EL under the highest temperature observed in this current study might thus be explained by the sampling timing after the recovery period.

The effect of abiotic stress on leaf nutrient concentration tends to be nutrient-specific as well as highly dependent on the stress intensity (Soares et al., 2019). Interestingly, leaf calcium concentration decreased under water stress in this current study despite a range of studies previously reporting an increase in other *Solanum* crops (Matìas et al., 2021; Pimenta et al., 2022; Sarker and Oba, 2018). While an increase in leaf calcium concentration in certain plant parts is important for the regulation of stress responses, a reduced soil water potential can reduce calcium uptake, as was observed for leaf phosphorus concentration (Ahanger et al., 2016). In field conditions, leaf phosphorus concentration increased under a temperature rise in quinoa (*Chenopodium quinoa* Willd.) as observed in this study (Matìas et al., 2021). Despite drought and heat having antagonist effects on this nutrient alone, no interactive effect was noted in this study with the maintained increase in leaf phosphorus concentration at T37 under deficit irrigation. Pathways used under each stress to influence leaf phosphorus levels might thus be isolated from each each other. The maintenance of leaf magnesium concentration under different temperature treatments in this current study is consistent with the observation made in quinoa under heat (Matìas et al., 2021). The decrease noted under water deficit was, however, not per previous results in tomato where no effect of drought was recorded (Pimenta et al., 2022). Adequate leaf magnesium concentration plays an important role in chlorophyll production, and plant growth maintenance, and has been previously shown to be highly

beneficial in heat stress tolerance by promoting a range of secondary metabolism pathways (Siddiqui et al., 2018). The interaction of stresses, in this case, did not impact the response of leaf magnesium concentration, highlighting that pathways in place to maintain leaf magnesium uptake under different temperatures were preserved under limited irrigation but also that no pathways to increase its levels to maintain heat stress tolerance under drought are present. A similar observation was made for leaf calcium concentration.

Micronutrients can have a beneficial effect on stress responses, for example, iron can reduce oxidative stress (Kumari et al., 2022). Under heat stress, a decrease in leaf iron concentration was noted, however, when water stress was also present, the opposite was true. Grain iron concentration also increased under the combination of heat and drought in chickpeas despite a decrease under heat alone due to protein denaturation (Benali et al., 2023). Protective mechanisms in place under drought might have offered protection to enzymes and proteins necessary for iron uptake, leading to an increase in iron uptake under drought and heat. In a study in sorghum (*Sorghum bicolor* L.), drought altered the translocation pathways of iron with seed and leaf iron increasing and decreasing, respectively (Araki et al., 2022). Translocation changes might thus be in place in the current study, with drought tolerance mechanism promoting leaf iron to a higher extent when plants are also under heat. As iron uptake is negatively impacted by high levels of soil phosphorus, manganese, and zinc, the dynamics of these minerals under stress will influence iron levels, adding complexity when trying to understand its dynamics (Ahanger et al., 2016). The leaf manganese concentration increase in T37 under both full and deficit irrigation shows a sustained heat response mechanism. Manganese has previously been suggested to help with photosynthesis, respiration, and nitrogen assimilation (Kumari et al., 2022). Leaf manganese concentration was also noted to increase in Amaranth under heat, but not Pak Choi, highlighting species differences (Hwang, Chao, and Lin, 2018). A decrease in leaf manganese due to water stress was only noted at T37, however, showing a lowered heat stress response when drought is also present.

5. Conclusion

This study aimed to describe the combined effects of drought and high temperature with a recovery period on the African eggplant Shum. Drought stress negatively impacted leaf and stem production, while moderate heat promoted plant growth. Chlorophyll levels were increased under water stress while they were affected by heat only during the heatwave period and were back to non-heated levels after a recovery period. The simultaneous application of drought and high temperature led to a similar effect to drought applied alone in plant growth and chlorophyll levels, showing its predominant effect on major plant characteristics. Water stress mostly affected the morphology and physiology of the plant but some mineral elements were also impacted by this stress. High temperatures affected the plant's primary and secondary metabolisms to different extend, with an increase in carbohydrates and boron only for well-watered plants under heat stress.

The results observed here support the successful vegetative development of the African eggplant under high temperatures and suggest their potential use during moderate heat waves with irrigation. The lack of rainfall and irrigation is more detrimental than high temperatures in this case and need to be considered in agricultural settings.

The African eggplant does not seem to activate its non-enzymatic antioxidative response pathways under drought and heat at these intensities. Further research on different growth stages and without a recovery period should be done to understand the dynamics throughout the stress period. Future research could also integrate field experiments to ensure the relevance of the observations for farmers. The effects of drought and heat, in particular their combination, on flowering, fruiting and yield would also be interesting to investigate to ensure farmers can maintain seed harvest from this leafy vegetable.

Author statement

The authors declare no conflicts of interest. The authors have worked together to develop the experiment, conduct it, write and revise the manuscript.

CRediT authorship contribution statement

David-Rogeat Noémie: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **Broadley Martin R.:** Conceptualization, Project administration, Supervision, Writing – review & editing. **Stavridou Eleftheria:** Conceptualization, Supervision, Writing – review & editing, Project administration.

Declaration of Competing Interest

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

Data availability

All data created during this research are openly available from the University of Nottingham data repository at https://doi.org/10.17639/nott.7383.

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