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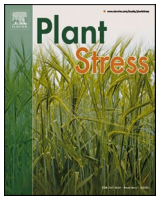

Exploring edamame survival mechanisms under combined drought and heat stress: Photosynthesis efficiency and carbohydrate accumulation

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ABSTRACT

The combined drought and heat (DH) stress have devastating effects on plant physiological and biochemical processes, leading to poor yield. The aims of this study were to identify the physio-biochemical mechanisms employed by edamame (*Glycine* max L. Merrill) for survival and to establish better performing cultivars under DH stress. The impact of DH stress on the photosynthesis efficiency and osmolytes production in three edamame cultivars (UVE14, UVE17 and AGS429) was investigated. Non-destructive measurements were performed to determine the photosynthesis attributes, while pigments, non-structural carbohydrates (starch, glucose, sucrose, trehalose) and proline were extracted and quantified spectrophotometrically. The results showed that chlorophyll *a* (Chl-a), Chl-b, total chlorophyll and carotenoids in AGS429 were not affected under DH stress, which corresponded to increased normalised difference vegetative index (NDVI). Positive correlations between the carotenoids and total chlorophyll contents suggest that in AGS429, prevention of chlorophyll degradation under DH stress could be attributed to the increased carotenoids because they have antioxidative function. Additionally, AGS429 and UVE14 had increased trehalose, suggesting high osmotic adjustment under DH stress. An increase in starch production maintained glucose balance in AGS429 and UVE14, demonstrating higher photosynthetic efficiency compared to UVE17. The DH stress reduced photochemical reactions and carbohydrate accumulation in UVE17. This study shows that AGS429 and UVE14 protected the photosystems and photosynthetic pigments during DH stress, which led to higher photosynthetic capacity and accumulation of carbohydrates. Thus, maintaining the photosynthesis efficiency and carbohydrate metabolism processes in the AGS429 and UVE14 were adaptation features under DH stress.

Introduction

Edamame (*Glycine* max L. Merrill), also known as large-seeded or vegetable-type soybean is a highly nutritious, water-demanding crop that is cultivated all over the world, including Africa (Mahoussi et al., 2020; Chen et al., 2022; Nair et al., 2023). Future temperature predictions indicate an expected 2 – 3 °C rise by 2050, which could cause severe drought and heat stress in plants (Tzortzakis et al., 2020; Kim and Kim, 2021), implying that drought and heat stress could co-exist under natural conditions (Abdelhakim et al., 2022). Combined drought and heat (DH) stress can have devastating effects on plants, impacting chlorophyll content, photosynthetic capacity, flower development, grain number, grain weight, and potentially causing plant death in severe cases (Laddomada et al., 2021). These effects can ultimately limit crop productivity, leading to high-yield losses and increased food prices (Mathobo et al., 2017). Ergo et al. (2018) demonstrated that DH stress during the pod-filling stage in grain-type soybeans significantly reduced yields, with physiological and biochemical traits accounting for 82 % of the observed variations. Additionally, DH stress led to chlorophyll degradation in *Podocarpus henkeli* and *Podocarpus falcatus* (Twala et al., 2022) and reduced the overall rate of photosynthesis in wheat (El Habti et al., 2020). While protection of the photosynthetic apparatus under DH stress helps in maintaining the energy fluxes per reaction centre (RE_0/RC), the probability of electron transfer to reduce end electron

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acceptors at photosystem I (PSI) [$\delta(R_0)$] decreases, leading to reduced synthesis of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) (Posso et al., 2020). Such findings highlight the critical need to understand the physiological and biochemical mechanisms in plants under DH stress. However, current knowledge remains limited as most studies have focused on the effects of drought or heat stress separately, thus indicating the need to investigate plants' (including edamame) physio-biochemical responses to combined drought and heat stress.

Exposure of plants to heat or drought may impact the photosynthesis efficiency, osmolytes accumulation, plant life cycle and growth (Zhao et al., 2021). Although stomatal closure contributes to the prevention of water loss in drought-stressed plants, it leads to reduced plant growth and development (Laurie et al., 2014; Mathobo et al., 2017). Under heat stress, open stomata prevent leaves from overheating, thereby promoting photosynthesis (Abdelhakim et al., 2022), while closing of the stomata under drought stress reduces the rate of photosynthesis. Shanmugam et al. (2013) demonstrated that exposure of wheat to heat stress increased the rate of photosynthesis. In contrast, heat stress significantly reduced chlorophyll *a* and *b* contents in grain-type soybean (Ortiz et al., 2022). Drought stress reduced the photosynthesis efficiency in susceptible edamame without significantly affecting chlorophyll content (Hlahla et al., 2022). However, carotenoids, which are accessory pigments in photosynthesis and excellent antioxidants that protect the photosynthetic apparatus from oxidative stress, were found to be less sensitive to drought and heat stress in sweet potato (Kim et al., 2021). In edamame, carotenoids were also positively associated with drought stress tolerance (Hlahla et al., 2022). Additionally, they are involved in the non-photochemical quenching (NPQ) of excess light in the photosystem II (PSII) antenna (Song et al., 2013).

During photosynthesis, two key carbohydrates (starch and glucose) involved in a plant's energy generation through complex metabolic processes are produced in the chloroplast and cytosol. Starch and sucrose hydrolysis leads to glucose production, which can join the cellular metabolism through the glycolytic pathway or synthesis of other molecules via the oxidative pentose phosphate (OPP) pathway. Additionally, glucose, trehalose, and sucrose, play a crucial role in drought tolerance processes, e.g., mono or disaccharides are involved in osmotic adjustment and antioxidative defence (Mohammadkhani and Heidari, 2008; Das et al., 2017), and starch acts as a reservoir that replenishes cellular mono or disaccharide. During pod filling in edamame, drought stress induced significant increases in the contents of these carbohydrates, including trehalose (Hlahla et al., 2022). Additionally, osmolytes such as proline have important antioxidative functions and cell osmotic adjustment during drought stress, making them key biochemical markers of drought tolerance. Moloi and van der Merwe (2021) demonstrated that proline content significantly increased in the edamame cultivars that were drought tolerant, validating its function as a maker for drought tolerance.

This study investigates the effects of combined drought and heat (DH) stress on photosynthetic and nonstructural carbohydrates physio-biochemical function in three edamame cultivars, i.e. AGS429, UVE14, and UVE17. Given the changing climate and the scarcity of studies on DH stress in edamame research, this study shows for the first time that stabilising and maintaining photosystems and photosynthetic pigments led to improved efficient photosynthesis and increased accumulation of glucose, sucrose, and trehalose in AGS429, and UVE14 treated with combined stress. Proline was increased in all stressed cultivars, which indicated that although this osmolyte has an important physiological role, it cannot be used to distinguish combined-stress tolerant cultivars (AGS429 and UVE14) from sensitive ones (UVE17).

Materials and methods

Plant material

Edamame seeds were originally obtained from Edamame Development Program (Republic of South Africa). The seeds were germinated in petri-dishes in the laboratory at 20 °C and transferred to seedling trays (after radicle emergence) containing hygromix seedling mix (Hygrotech, RSA). At unifoliate leaf stage, the seedlings were transplanted in potting bags containing red sandy-loamy soil in the glasshouse (29°6′30.8″ S; 26°11′19.4″ E) at the Bloemfontein campus of the University of the Free State (UFS, Republic of South Africa). The cultivars used were AGS429 (moderately drought tolerant, high yielding), UVE14 (drought tolerant, low yielding), and UVE17 (drought susceptible). The growth conditions up to the second trifoliate leaf stage (V2) were set at 25 °C during the day and 18 °C at night with plants irrigated to 100 % soil water holding capacity (WHC). At the third trifoliate leaf stage, plants were subjected to a combination of drought (30 % WHC) and high temperature stress (35 °C during the day and 27 °C at night). Positive control plants were continuously subjected to irrigation at 100 % WHC at 25 °C and 18 °C (day and night). The negative control plants were subjected to either drought stress (30% WHC) or high temperature stress (35 °C, day and 27 °C, night) only. The experimental design was split-split-plot randomized design; where the main plot was temperature, the sub-plot was water treatment, and the sub-subplot was the cultivar. Three replications per treatment were used to increase statistical validation of the results. Sampling, both destructive and non-destructive, was conducted on young, fully expanded trifoliate leaves during the flowering and pod-filling stages. This timing is critical for assessing the effects of DH stress because flower and pod development demands significant energy and carbon skeletons from photosynthesis. Sufficient carbohydrate availability during these stages ensures the proper formation and growth of these reproductive structures, promoting successful reproduction and seed development. The leaves were crushed in liquid nitrogen after sampling and stored at −26 °C.

Chlorophyll fluorescence

A portable photosynthesis efficiency analyser (PEA) fluorimeter (Hansatech instrument, King's Lynn, UK) was used to measure the potential quantum efficiency of photosystem II (PSII) and photosystem I (PSI) according to Pareek et al. (2019). The fluorescence measurements were performed during summer season (October to December) between 10:00 and 12:00 at flowering and pod filling growth stages. The leaves were dark adapted for 30 min and illuminated with excitation light energy set at 3500 μmol/m^2s. The ratio of variable fluorescence to maximal fluorescence of PSll (Fv/Fm), performance index of PSII and PSI (PI_{ABS}), total performance index of PSII and PSI (PI_{Total}), energy absorbed per reaction centre (RC) (ABS/RC), energy dissipated as heat per RC (DI_0/RC), trapped energy per RC (TR_0/RC), the flux of electrons transferred from quinone (Q_A^-) to plastoquinone (PQ) per active PSII per RC (ET_0/RC), electron flux reducing end electron acceptors at PSl acceptor side per RC (RE_0/RC), efficiency/probability that an electron moves further than Q_A^- ($\psi(E_0)$), and the efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSl acceptor side (δR_0) were measured to monitor the photosynthetic efficiency of the three edamame cultivars. The normalised difference vegetation index (NDVI) and photochemical reflectance index (PRI) were measured using a leaf spectrometer (SpectraVue, CID Bio-Science, Camas, USA).

Stomatal conductance

The stomatal conductance on the leaves was measured at flowering and pod filling stages using a leaf porometer (Li-Cor ADC BioScientific Ltd., Hoddensdon, UK). The stomatal conductance was performed

Table 1
The photosynthesis pigments of three edamame cultivars under combined drought and heat (DH) stress at flowering stage.

Cultivar	Treatment	Chl-a (mg/g fw)	Chl-b (mg/g fw)	CRD (mg/g fw)	Chl_T (mg/g fw)	NDVI
AGS429	Control	26.9 ± 2.2^{a}	25.3 ± 3.1^{cd}	10.7 ± 1.1^{abc}	62.5 ± 4.2^{abc}	0.633 ±0.1^{a}
	Drought	27.1 ± 4.0^{a}	22.7 ± 2.7^{bcd}	10.5 ± 0.7^{abc}	61.5 ± 6.6^{abc}	0.702 ±0.03^{ab}
	Heat	25.5 ± 1.2^{a}	12.8 ± 3.3^{a}	9.5 ± 1.4^{a}	53.2 ± 5.6^{ab}	0.675 ±0.09^{ab}
	DH	26.8 ± 1.3^{a}	25.1 ± 3.0^{cd}	10.6 ± 0.8^{abc}	62.3 ± 4.3^{abc}	0.989 ±0.1^{b}
UVE14	Control	26.9 ± 2.4^{a}	26.4 ± 2.2^{d}	10.7 ± 1.6^{abc}	63.2 ± 3.6^{abc}	0.711 ±0.06^{ab}
	Drought	26.7 ± 1.3^{a}	28.8 ± 1.3^{d}	10.8 ± 0.2^{abc}	64.2 ± 5.1^{abc}	0.692 ±0.08^{ab}
	Heat	25.4 ± 1.0^{a}	15.9 ± 0.9^{ab}	10.0 ± 1.1^{ab}	53.9 ± 2.2^{ab}	0.683 ±0.04^{ab}
	DH	25.6 ± 1.9^{a}	14.3 ± 1.7^{ab}	9.4 ± 0.6^{a}	51.1 ± 4.8^{a}	0.680 ±0.07^{ab}
UVE17	Control	26.9 ± 2.1^{a}	24.8 ± 2.4^{cd}	10.5 ± 0.9^{abc}	62.2 ± 3.4^{abc}	0.705 ±0.2^{ab}
	Drought	27.2 ± 3.1^{a}	23.0 ± 1.5^{bcd}	10.5 ± 2.3^{abc}	61.7 ± 3.4^{abc}	0.707 ±0.06^{ab}
	Heat	27.1 ± 2.3^{a}	21.4 ± 1.1^{abcd}	10.5 ± 1.8^{abc}	60.7 ± 2.6^{abc}	0.689 ±0.08^{ab}
	DH	26.9 ± 1.5^{a}	16.6 ± 1.5^{abc}	9.5 ± 0.8^{a}	57.7 ± 3.2^{ac}	0.672 ±0.09^{ab}

Control = 100% WHC + 25/18 °C, Drought = 30% WHC + 25/18 °C, Heat = 100% WHC + 35/27 °C, DH = 30% WHC + 35/27 °C, Values represent means ± SD (n = 3), different alphabets represent significant differences between treatments ($p \leq 0.05$) in Chl-a = Chlorophyll *a*, Chl-b = Chlorophyll *b*, CRDs = Carotenoids and Chl_T= Total chlorophyll, and NDVI = normalised difference vegetation index. AGS429 = High yielding with moderate yield reduction under drought stress, UVE14 = low yielding with low yield reduction under drought stress, UVE17 = low yielding with high yield reduction under drought stress.

Table 2
The photosynthetic pigments contents for three edamame cultivars under combined drought and heat (DH) stress at pod filling stage.

Cultivar	Treatment	Chl-a (mg/g fw)	Chl-b (mg/g fw)	CRD (mg/g fw)	Chl_T (mg/g fw)	NDVI
AGS429	Control	13.4 ± 2.1^{a}	27.2 ± 2.4^{c}	5.6 ± 0.6^{a}	48.2 ± 3.04^{abc}	0.712 ±0.04^{a}
	Drought	16.5 ± 1.3^{ab}	19.5 ± 1.9^{abc}	6.7 ± 0.2^{ab}	40.5 ± 3.8^{ab}	0.701 ±0.08^{a}
	Heat	20.4 ± 2.1^{abc}	23.6 ± 2.2^{ac}	7.9 ± 0.5^{ab}	49.9 ± 2.7^{abc}	0.717 ±0.07^{a}
	DH	19.8 ± 1.8^{abc}	23.3 ± 0.6^{ac}	7.9 ± 0.8^{ab}	48.7 ± 2.3^{abc}	0.698 ±0.1^{a}
UVE14	Control	17.1 ± 0.7^{ab}	12.6 ± 1.1^{a}	6.1 ± 0.6^{ab}	37.8 ± 1.8^{ab}	0.710 ±08^{a}
	Drought	18.5 ± 1.5^{ab}	26.6 ± 2.7^{c}	7.1 ± 0.4^{ab}	47.1 ± 2.1^{abc}	0.697 ±0.1^{a}
	Heat	21.1 ± 1.8^{abc}	23.1 ± 2.0^{ac}	9.5 ± 0.6^{b}	50.9 ± 4.3^{abc}	0.707 ±0.08^{a}
	DH	20.1 ± 1.1^{abc}	21.8 ± 1.5^{ac}	7.9 ± 0.8^{ab}	48.4 ± 3.3^{abc}	0.681 ±0.05^{a}
UVE17	Control	14.2 ± 1.3^{a}	16.4 ± 1.3^{ab}	6.8 ± 0.3^{ab}	34.8 ± 2.3^{a}	0.708 ±0.1^{a}
	Drought	15.9 ± 1.9^{ab}	19.8 ± 1.0^{abc}	6.7 ± 0.9^{ab}	39.8 ± 3.7^{ab}	0.708 ±0.05^{a}
	Heat	24.0 ± 2.03^{c}	25.6 ± 2.2^{c}	9.3 ± 1.0^{b}	55.8 ± 4.7^{c}	0.723 ±0.06^{a}
	DH	18.5 ± 1.6^{ab}	19.6 ± 1.8^{abc}	7.8 ± 0.9^{ab}	44.2 ± 4.08^{abc}	0.706 ±0.07^{a}

Control = 100% WHC + 25/18 °C, Drought = 30% WHC + 25/18 °C, Heat = 100% WHC + 35/27 °C, DH = 30% WHC + 35/27 °C, Values represent means ± SD (n = 3), different alphabets represent significant differences between treatments ($p \leq 0.05$) in Chl-a = Chlorophyll *a*, Chl-b = Chlorophyll *b*, CRDs = Carotenoids and Chl_T= Total chlorophyll, and NDVI = normalised difference vegetation index. AGS429 = High yielding with moderate yield reduction under drought stress, UVE14 = low yielding with low yield reduction under drought stress, UVE17 = low yielding with high yield reduction under drought stress.

during summer season (October to December) between 10:00 and 13:00.

Extraction and measurements of photosynthesis pigments

Frozen, powdered leaf tissue (100 mg) was homogenised in 2 mL of 80 % (v/v) chilled acetone (Sigma-Aldrich, Saint Louis, MO, USA). The homogenate was centrifuged at 5000 x *g* for 5 min at 4 °C and the supernatant was used to spectrophotometrically quantify chlorophyll *a* (Chl-a), chlorophyll *b* (Chl-b), total chlorophyll and carotenoid (CRDs) contents. The content of the different pigments was calculated according to Pareek et al. (2019) and expressed in mg/g fresh weight.

Extraction and quantification of soluble and stored carbohydrates

Soluble carbohydrates (glucose, sucrose and trehalose) and starch were extracted according to Zhao et al. (2010). Frozen, powdered leaf tissue (100 mg) was extracted twice with 2 mL of 80 % (v/v) ethanol (Sigma-Aldrich, RSA, Saint Louis, MO, USA). The extract was boiled at 80 °C for 15 min, followed by centrifugation at 5000 x *g* for 5 min. To remove other non-sugar leaf metabolites, 60 mg of activated charcoal (Merck, Darmstadt, Germany) was added to the 4 mL supernatant, followed by incubation for 5 min at room temperature and centrifugation at 3000 x *g* for 10 min.

Starch was extracted from the pellet left in the tubes after soluble carbohydrates extraction according to Zhao et al. (2010). Ethanol (0.2 mL, 80 %, v/v) was added to the tubes and vortexed to aid dispersion, followed by the addition of 2 mL of 2 M KOH (BDH Chemicals, England). The tubes were then boiled in a water bath at 100 °C for 1 h, while gently stirring every 10 min. After the boiling, the tubes were allowed to cool at room temperature before the addition of 8 mL of 1.2 M sodium acetate buff (pH 3.8). The tubes were centrifuged at 3000 x *g* for 10 min, and the supernatant was used to determine starch content using a total starch HK assay kit (Megazyme, Wicklow, Ireland).

Proline extraction and quantification

Proline was extracted from 0.3 g leaf tissue samples according to Carillo and Gibon (2011). Four (4) mL ethanol (70 %, v/v) was added to the leaf tissue (300 mg) and homogenised to a fine paste. The tubes were centrifuged at 3000 x *g* for 10 min. Ethanol (20 %, v/v), 500 µL) and 500 µL of 1 % (w/v) ninhydrin in 60 % (v/v) glacial acetic acid were added to 500 µL extract. The mixture was vortexed and incubated at 95 °C for 20 min. The samples were then centrifuged at 10,000 x *g* for 10 min before measuring the absorbance at 520 nm against the blank. Similar steps were followed to make a proline standard, which was prepared by making a serial dilution of 0.016 – 0.16 mM proline in 70 % (v/v) ethanol.

Statistical analysis

Statistical analysis was performed using Statistica Release 7 software. The Shapiro–Wilk normality test was used to test for normality of the collected data. The separate and combined effects of temperature, water treatment, and cultivar were determined using the analysis of variance (ANOVA). The Fischer's protected least significant differences (LSD) test at the alpha value of $p = 0.05$ was used to separate the means. The positive and negative associations between the photosynthesis and osmolytes parameters were calculated using Pearson's correlations.

Results

Table 1 represents the impact of DH stress on the photosynthesis

Table 3
The combined drought and heat (DH) stress impact on the light-dependant reactions in three edamame cultivars at the flowering stage.

Cultivar	Treatment	PRI	F_v/F_m	ABS/RC	DI_0/RC	TR_0/RC	ET_0/RC	RE_0/RC	$\Psi(E_0)$	$\delta(R_0)$	PI_{ABS}	PI_{Total}
AGS429	Control	0.034 ±0^{ab}	0.76 ±0.02^{b}	1.73 ±0.1^{a}	0.42 ±0.01^{a}	1.31 ±0.3^{ab}	0.47 ±0.03^{a}	0.30±0.01^{a}	0.36 ±0.03^{ab}	1.29 ±0.09^{ab}	1.32 ±0.02^{abc}	1.83 ±0.04^{a}
	Drought	0.033 ±0^{ab}	0.76±0^{b}	1.73 ±0.06^{a}	0.43 ±0.2^{a}	1.30 ±0.1^{ab}	0.44 ±0.1^{a}	0.26±0.06^{a}	0.34 ±0.01^{ab}	0.60 ±0.01^{a}	1.23 ±0.08^{abc}	1.65 ±0.03^{a}
	Heat	0.034 ±0^{ab}	0.58 ±0.03^{ab}	1.60 ±0.08^{a}	0.85 ±0.1^{a}	1.45 ±0.4^{ab}	1.01±02^{a}	1.88±0^{bc}	0.56 ±0.01^{b}	1.18 ±0.01^{ab}	0.39 ±0.05^{ab}	1.60 ±0.03^{a}
	DH	0.027 ±0^{a}	0.54 ±0.01^{a}	1.52 ±0.2^{a}	0.57 ±0.2^{a}	1.05±0.2^{a}	0.48 ±0.02^{a}	0.81±0.1^{ab}	1.43 ±0.08^{bc}	1.86 ±0.07^{b}	0.30 ±0.01^{a}	1.63 ±0.05^{a}
UVE14	Control	0.033 ±0^{ab}	0.78 ±0.01^{b}	1.57 ±0.1^{a}	0.56 ±0.2^{a}	1.21 ±0.1^{ab}	1.50 ±0.1^{a}	0.31±0.01^{a}	0.42 ±0.05^{ab}	0.63 ±0.03^{a}	2.12±0.2^{c}	3.60 ±0.02^{a}
	Drought	0.033 ±0^{ab}	0.78 ±0.03^{b}	1.69 ±0.3^{a}	0.58 ±0.08^{a}	1.31 ±0.2^{ab}	1.51 ±0.1^{a}	0.30±0.07^{a}	0.39 ±0.02^{ab}	0.61 ±0.03^{a}	1.72 ±0.08^{abc}	2.48 ±0.3^{a}
	Heat	0.028 ±0^{a}	0.68 ±0.02^{ab}	2.03 ±0.3^{ab}	0.61 ±0.3^{a}	1.46 ±0.07^{ab}	1.57 ±0.05^{a}	0.54±0.05^{a}	0.36 ±0.1^{ab}	1.03 ±0.05^{ab}	0.82 ±0.06^{bc}	8.60 ±1.9^{b}
	DH	0.029 ±0^{a}	0.62 ±0.02^{ab}	1.74 ±0.3^{a}	0.51 ±0.2^{a}	1.23 ±0.4^{ab}	1.51 ±0.09^{a}	3.61±0.05^{c}	0.44 ±0.1^{ab}	1.10 ±0.05^{ab}	0.79 ±0.04^{bc}	3.99 ±0.7^{a}
UVE17	Control	0.037 ±0^{b}	0.77 ±0.03^{b}	1.57 ±0.07^{a}	0.37 ±0.04^{a}	1.19 ±0.2^{ab}	0.50 ±0.05^{a}	0.32±0.03^{a}	0.43 ±0.08^{ab}	0.65 ±0.03^{a}	1.78 ±0.1^{abc}	3.35 ±0.9^{a}
	Drought	0.034 ±0^{ab}	0.78 ±0.05^{b}	1.56 ±0.1^{a}	0.35 ±0.3^{a}	1.21 ±0.2^{ab}	0.46 ±0.03^{a}	0.25±0.03^{a}	0.39 ±0.06^{ab}	0.58 ±0.02^{a}	1.99 ±0.3^{abc}	2.30 ±0.3^{a}
	Heat	0.033 ±0^{ab}	0.60 ±0.02^{ab}	2.20 ±0.2^{b}	1.46 ±0.1^{b}	1.34 ±0.06^{ab}	0.51 ±0.02^{a}	0.75 ±0.0.03^{ab}	0.29 ±0.04^{a}	1.40 ±0.06^{ab}	0.83 ±0.06^{bc}	3.26 ±0.8^{a}
	DH	0.027 ±0^{a}	0.53 ±0.03^{a}	1.95 ±0.6^{ab}	1.11 ±0.2^{ab}	1.84 ±0.08^{b}	0.46 ±0.06^{a}	1.18±0.07^{b}	0.25 ±0.01^{a}	1.68 ±0.02^{b}	0.28 ±0.03^{a}	3.46 ±0.5^{a}

Control = 100% WHC + 25/18 °C, Drought = 30% WHC + 25/18 °C, Heat = 100% WHC + 35/27 °C, DH = 30% WHC + 35/27 °C, Values represent means± SD (n = 3), different alphabets represent significant differences between treatments ($p \leq 0.05$) in Fv/Fm = Ratio of variable fluorescence to maximal fluorescence of PSII, PI_{ABS} = Performance index of photosystem I (PSI) and photosystem II (PSII), PI_{Total} = Total performance index of PSI and PSII, ABS/RC = Energy absorbed per reaction centre (RC), DI_0/RC = Energy dissipated as heat per RC, TR_0/RC = trapped energy per RC, ET_0/RC = the flux of electrons transferred from quinone (Q_A^-) to plastoquinone (PQ) per active PSII per RC, RE_0/RC = electron flux reducing end electron acceptors at PSI acceptor side per RC, $\psi(E_0)$ = efficiency/probability that an electron moves further than Q_A^-, and $\delta(R_0)$ = efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side, and PRI = Photochemical reflectance index. AGS429 = High yielding with moderate yield reduction under drought stress, UVE14 = low yielding with low yield reduction under drought stress, UVE17 = low yielding with high yield reduction under drought stress.

Table 4
The combined drought and heat (DH) stress impact on the light-dependant reactions in three edamame cultivars at pod filling stage.

Cultivar	Treatment	PRI	F_v/F_m	ABS/RC	DI_0/RC	TR_0/RC	ET_0/RC	RE_0/RC	$\Psi(E_0)$	$\delta(R_0)$	PI_{ABS}	PI_{Total}
AGS429	Control	0.024 ±0^{ab}	0.82 ±0.02^{ab}	1.58 ±0.03^{b}	0.31±0.1^{b}	1.27 ±0.01^{cd}	0.53 ±0.02^{abcd}	0.28 ±0.01^{ab}	0.43 ±0.05^{ab}	0.53 ±0.05^{cdef}	2.17 ±0.6^{a}	3.31 ±0.9^{ab}
	Drought	0.020 ±0^{ab}	0.83 ±0.09^{b}	1.19 ±0.03^{bc}	0.21 ±0.03^{a}	0.98 ±0.04^{ab}	0.59 ±0.04^{acde}	0.30 ±0.03^{ab}	0.61 ±0.03^{c}	0.50 ±0.06^{abc}	7.26 ±1.4^{c}	6.99 ±1.3^{de}
	Heat	0.026 ±0^{b}	0.82 ±0.1^{ab}	1.61 ±0.01^{b}	0.31 ±0.05^{b}	1.30±0.03^{d}	0.66±0.03^{de}	0.25 ±0.04^{a}	0.52 ±0.08^{abc}	0.49 ±0.05^{abc}	3.52 ±0.8^{ab}	2.21 ±0.8^{a}
	DH	0.023 ±0^{ab}	0.83 ±0.08^{b}	1.51 ±0.03^{ac}	0.28 ±0.03^{abc}	1.23 ±0.05^{bcd}	0.70±0.04^{e}	0.25 ±0.02^{a}	0.57 ±0.05^{bc}	0.36 ±0.06^{a}	4.07 ±1.1^{abc}	3.33 ±0.8^{ab}
UVE14	Control	0.026 ±0^{b}	0.79 ±0.06^{a}	1.44 ±0.04^{abc}	0.31 ±0.02^{b}	1.13 ±0.02^{abcd}	0.44±0.03^{a}	0.29 ±0.03^{ab}	0.41 ±0.04^{a}	0.66±0.1^{f}	2.34 ±0.8^{a}	4.16 ±1.1^{abc}
	Drought	0.023 ±0^{ab}	0.82 ±0.08^{ab}	1.24 ±0.03^{ab}	0.23 ±0.09^{ab}	1.01 ±0.03^{abc}	0.51 ±0.05^{abc}	0.28 ±0.03^{ab}	0.52 ±0.04^{abc}	0.58 ±0.05^{def}	4.83 ±0.6^{abc}	6.40 ±1.6^{cde}
	Heat	0.030 ±0^{b}	0.82 ±0.05^{ab}	1.42 ±0.03^{abc}	0.28 ±0.05^{abc}	1.14 ±0.03^{abcd}	0.62 ±0.03^{cde}	0.27 ±0.04^{ab}	0.54 ±0.02^{abc}	0.54 ±0.05^{cdef}	3.65 ±0.9^{ab}	3.06 ±0.9^{ab}
	DH	0.027 ±0^{b}	0.83 ±0.03^{b}	1.45 ±0.06^{abc}	0.28 ±0.06^{abc}	1.17 ±0.05^{abcd}	0.67±0.07^{e}	0.33 ±0.01^{b}	0.58 ±0.02^{bc}	0.46 ±0.05^{ab}	4.24 ±0.8^{abc}	3.90 ±0.9^{abc}
UVE17	Control	0.022 ±0^{ab}	0.79 ±0.03^{a}	1.46 ±0.02^{abc}	0.31 ±0.04^{b}	1.15 ±0.07^{abcd}	0.47 ±0.06^{ab}	0.28 ±0.01^{ab}	0.43 ±0.01^{ab}	0.59 ±0.09^{def}	2.35 ±0.6^{a}	3.18 ±0.7^{ab}
	Drought	0.028 ±0^{b}	0.83 ±0.05^{b}	1.12±0.1^{a}	0.29 ±0.01^{abc}	0.93±0.05^{a}	0.56 ±0.03^{abcde}	0.30 ±0.05^{ab}	0.61 ±0.02^{c}	0.54 ±0.04^{cdef}	6.96 ±1.8^{bc}	8.16 ±1.4^{e}
	Heat	0.029 ±0^{b}	0.81 ±0.04^{ab}	1.44 ±0.05^{abc}	0.28 ±0.06^{abc}	1.15 ±0.1^{abcd}	0.65±0.05^{de}	0.25 ±0.04^{a}	0.57 ±0.06^{bc}	0.50 ±0.05^{abc}	4.90 ±0.8^{abc}	3.33 ±0.8^{ab}
	DH	0.015 ±0^{a}	0.82 ±0.07^{ab}	1.27 ±0.08^{ab}	0.23 ±0.03^{ab}	1.03 ±0.06^{abc}	0.64±0.05^{de}	0.28 ±0.05^{ab}	0.62 ±0.05^{c}	0.44 ±0.04^{ab}	6.29 ±0.8^{bc}	4.91 ±0.9^{bcd}

Control = 100% WHC + 25/18 °C, Drought = 30% WHC + 25/18 °C, Heat = 100% WHC + 35/27 °C, DH = 30% WHC + 35/27 °C, Values represent means± SD (n = 3), different alphabets represent significant differences between treatments ($p \leq 0.05$) in Fv/Fm = Ratio of variable fluorescence to maximal fluorescence of PSII, PI_{ABS} = Performance index of photosystem I (PSI) and photosystem II (PSII), PI_{Total} = Total performance index of PSI and PSII, ABS/RC = Energy absorbed per reaction centre (RC), DI_0/RC = Energy dissipated as heat per RC, TR_0/RC = trapped energy per RC, ET_0/RC = the flux of electrons transferred from quinone (Q_A^-) to plastoquinone (PQ) per active PSII per RC, RE_0/RC = electron flux reducing end electron acceptors at PSI acceptor side per RC, $\psi(E_0)$ = efficiency/probability that an electron moves further than Q_A^-, and $\delta(R_0)$ = efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side, and PRI = Photochemical reflectance index. AGS429 = High yielding with moderate yield reduction under drought stress, UVE14 = low yielding with low yield reduction under drought stress, UVE17 = low yielding with high yield reduction under drought stress.

pigments of three edamame cultivars at the flowering stage. In all cultivars, DH stress had an insignificant effect on Chl-a content compared to the optimal conditions (control, C) and individual treatment conditions, i.e., drought (D) or heat (H) treatment. Compared to the control, Chl-b content for AGS429 was not significantly affected by DH treatment; however, individual treatments inhibited Chl-b accumulation (10 % and 49 % reduction under D and H stress treatments). Chlorophyll *b* of UVE14 under DH treatment was substantially reduced compared to the control (45 %), D (50 %) and H (10 %) treatments. Although Chl-b was reduced under D and H stress compared to the control in UVE17, the DH treatment reduced the highest Chl-b content (33 %). The CRD content of DH treated AGS429 was not significantly different to that of the control and D treatments but was slightly higher (10 %) than that of H treatment. For UVE14 and UVE17, the most notable reduction in the CRD content was observed under DH treatment (12 % and 10 %, respectively). The total chlorophyll (Chl_T) content in AGS429 was not affected by DH treatment and was 15 % higher than that of H treatment. Although D alone had no significant effect on the total chlorophyll accumulation in UVE14, H and DH substantially reduced the parameter (15 % and 19 %, respectively). Compared to other treatments, NDVI was the highest in AGS429 under DH treatment. For UVE14 and UVE17, NDVI was not significantly different for all treatments.

Table 2 represents the impact of DH stress on the photosynthesis pigments of three edamame cultivars at pod filling stage. Compared to the control, Chl-a content increased substantially under individual or DH treatments in all cultivars. The Chl-b content in AGS429 under all treatments was slightly reduced compared to the control, while for other cultivars, a general increase was observed. Compared to the control, CRD content increased in AGS429, while it was the same for other cultivars under DH treatment. The total chlorophyll (Chl_T) content for AGS429 under DH treatment was not significantly different to that of H stress but was substantially high compared to the control (26 %) and D (17 %) treatments. For UVE14 and UVE17, Chl_T was significantly increased under all stress treatments compared to the controls.

Table 3 represents the impact of DH stress on the chlorophyll *a* fluorescence (OJIP transient parameters) and photochemical reflective index (PRI) of three edamame cultivars at flowering. Compared to all treatments, DH stress notably reduced PRI and Fv/Fm in AGS429 (21 % and 29 %, respectively) with the highest reduction in UVE17 (27 % and 31 %, respectively). Under DH treatment, ABS/RC, DI_0/RC, TR_0/RC, and RE_0/RC, were significantly high in UVE17. The $\Psi(E_0)$ and $\delta(R_0)$ for AGS429 were significantly high under DH compared to the control, D and H, but for UVE14 these parameters were not significantly different to that of the control, D and H. In UVE17, the (R_0) was higher than the control and D, but 17 % lower than H. The RE_0/RC in UVE14 was significantly higher than the control, D, and H (91 %, 92 % and 85 %, respectively). In AGS429, the RE_0/RC was significantly high under DH stress compared to the control (63 %). Although drought alone had no significant effect on PI_{ABS} in AGS429, DH treatment led to the highest reduction (77 %) compared to the control. Compared to the control and D in UVE17, the H and DH stress led to 84 % and 86 % reduction in PI_{ABS} respectively, and this cultivar had the lowest PI_{ABS} under DH stress compared to other cultivars at the flowering stage.

Table 4 represents the impact of DH stress on the chlorophyll *a* fluorescence (OJIP transient parameters) and PRI of three edamame cultivars at pod filling. Compared to the control and each stress separately, DH treatment had a substantial reduction impact on the PRI of only UVE17. The DH treatment resulted in reduced ABS/RC compared to the control and H treatment in AGS429. Compared to other cultivars under DH treatment, UVE17 had the lowest ABS/RC. However, DH had a lesser impact on this parameter than under D treatment alone. The DI_0/RC was not affected by any stress treatment in AGS429. For UVE14, there was a reduction in all stress treatments with DI_0/RC under DH not significantly different to that of D and H treatments alone. The highest reduction was in DH stressed UVE17. The TR_0/RC was reduced by combined DH in AGS429 and UVE17 compared to the control, and the separate D treatments. There was significantly high ET_0/RC, RE_0/RC, and $\psi(E_0)$ in UVE14 under DH stress compared to other treatments. The ET_0/RC was remarkably increased by the DH stress in AGS429 compared to the control (24 %), drought (16 %), and heat (6 %). The $\delta(R_0)$ was significantly reduced compared to other treatments in all cultivars under combined DH stress. The PI_{ABS} under DH stress was low compared to D treatment but significantly high compared to the control and H treatment in all cultivars. Drought treatment resulted in high PI_{Total} in all cultivars, but the DH treatment led to an insignificant PI_{Total} response compared to the control in AGS429 and UVE14. The DH treatment increased PI_{Total} in UVE17 significantly compared to the control (35 %) and H treatment (32 %), but this was 40 % lower than the D treatment. The DH stress significantly increased PI_{ABS} and PI_{Total} compared to the control and H treatment in AGS429 and UVE17.

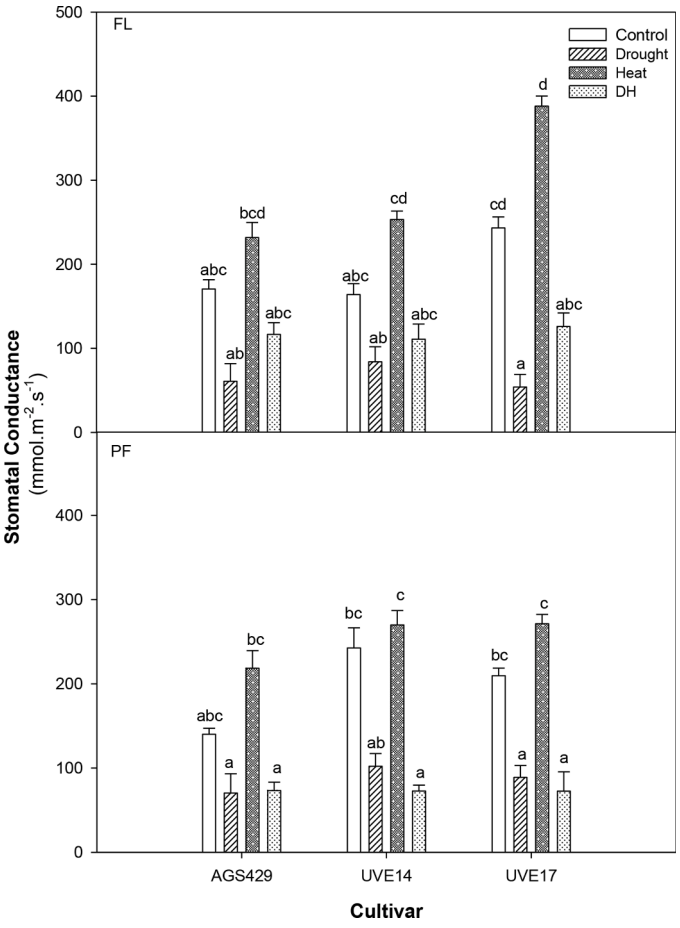

Fig. 1. Stomatal conductance of three edamame cultivars under drought, heat, combined drought and heat (DH) stress at flowering (FL) and pod filling (PF) stages. Alphabets represent similarities or differences in stomatal conductance between treatments and cultivars. Values represent means ±SD ($n = 3$). Control = 100% WHC + 25/18 °C, Drought = 30% WHC + 25/18 °C, Heat = 100% WHC + 35/27 °C, DH = 30% WHC + 35/27 °C. AGS429 = High yielding with moderate yield reduction under drought stress, UVE14 = low yielding with low yield reduction under drought stress, UVE17 = low yielding with high yield reduction under drought stress.

Fig. 1 shows results for the stomatal conductance (g_s) in three edamame cultivars under DH stress. Although the g_s was low under D and high under H stress for all cultivars irrespective of the growth stage, the DH treatment led to a significant ($p \leq 0.05$) reduction in UVE17 (48 %) compared to the control at flowering. Compared to D, DH stress at pod filling, DH stress significantly reduced the g_s in AGS429 (46 %), UVE14 (70 %) and UVE17 (66 %) compared to the control. The reduction in g_s was highest in UVE14 and UVE17 under DH treatment.

Fig. 2A shows the starch content of three edamame cultivars under

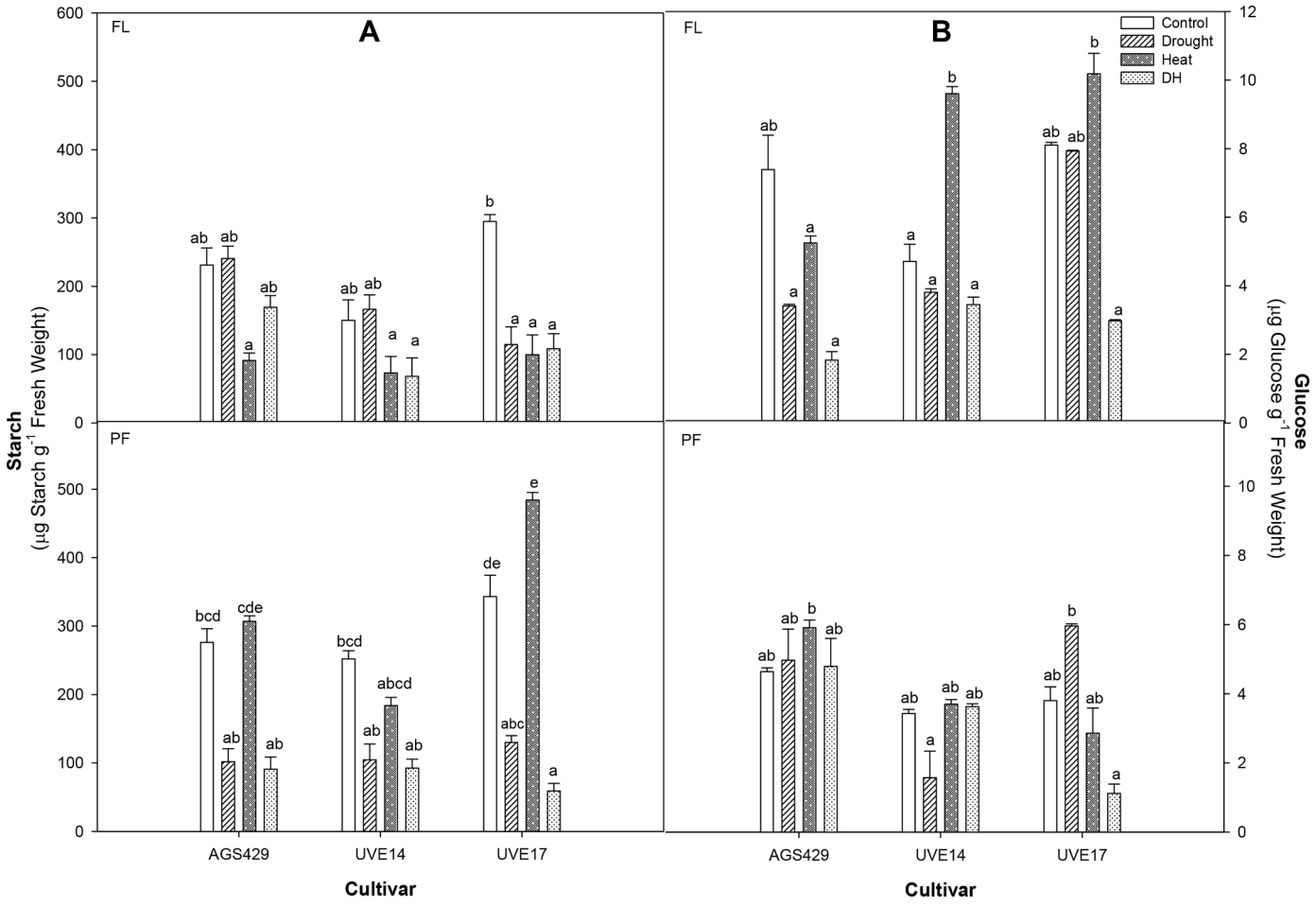

Fig. 2. Starch (A) and glucose (B) content of three edamame cultivars under drought, heat, combined drought and heat (DH) stress at flowering (FL) and pod filling (PF) stages. Alphabets represent similarities or differences in starch or glucose content between treatments and cultivars. Values represent means ± SD ($n = 3$). Control = 100% WHC + 25/18 °C, Drought = 30% WHC + 25/18 °C, Heat = 100% WHC + 35/27 °C, DH = 30% WHC + 35/27 °C. AGS429 = High yielding with moderate yield reduction under drought stress, UVE14 = low yielding with low yield reduction under drought stress, UVE17 = low yielding with high yield reduction under drought stress.

DH stress. There was low starch content in UVE14 at flowering under all treatments and significant reduction under DH treatment compared to the control (55 %) and drought (59 %). In UVE17, starch was significantly reduced under all treatments compared to the control ($p \leq 0.05$). Similarly, DH stress significantly reduced starch content in AGS429 (67 %), UVE14 (63 %), and UVE17 (85 %) compared to the control, with the lowest content in UVE17 under DH treatment at pod filling. The DH stress significantly inhibited glucose accumulation ($p \leq 0.05$) in AGS429 (75 %) and UVE17 (63 %) in relation to the control at flowering (Fig. 2B). At pod filling, the DH stress significantly reduced glucose content in UVE17 (71 %) compared to the control and 80 % compared to D treatment. This cultivar had the lowest glucose content under DH treatment at pod filling.

Fig. 3A represents the effects of DH stress on sucrose content in three edamame cultivars. The sucrose concentration in AGS429 and UVE17 was significantly reduced by DH stress compared to control (64 % and 71 %), D (43 % and 73 %) and H (56 % and 75 %) at the flowering stage, while UVE14 showed an insignificant accumulation of sucrose under DH treatment compared to the control and D treatment. The DH treatment slightly increased the sucrose content in all cultivars (though insignificant) at pod filling. Although H treatment induced trehalose accumulation in UVE14 and UVE17, the DH stress led to the highest trehalose accumulation in UVE14 (51 %) compared to the control at flowering stage (Fig. 3B). At pod filling, all treatments led to higher trehalose concentration compared to the control in UVE14, but DH stress resulted in the highest trehalose accumulation (84 %), compared to the control. The DH stress increased trehalose content by 42 % compared to the control in AGS429 at pod filling. The D treatment led to trehalose accumulation at both growth stages in UVE17, but DH stress had an insignificant effect on trehalose content at both growth stages in this cultivar.

Fig. 4 represents the proline content in three edamame cultivars under DH stress. The DH stress significantly ($p \leq 0.05$) increased proline content in AGS429 and UVE17 compared to the control (59 % and 65 %, respectively) and heat treatment (46 % and 53 %, respectively) at flowering stage. At the pod filling stage, DH stress significantly led to proline accumulation in AGS429 compared to the control and D treatment (60 % and 43 %, respectively). Although proline accumulation was substantially upregulated in UVE14 under DH treatment (48 % compared to the control) at pod filling, there were no significant differences between DH and H or D treatments. The accumulation of proline in UVE17 at the pod filling stage was insignificant under all treatments, but AGS429 maintained significantly high proline content under DH stress.

Tables 5 and 6 represent the correlations between the photosynthesis and osmolyte parameters of three edamame cultivars under DH stress. There was a strong significant positive correlation between the ABS/RC, TR_0/RC, ET_0/RC, RE_0/RC and sucrose at the flowering stage. The CRDs positively correlated with Chl-a, Chl-b and total chlorophyll (Chl_T). Glucose negatively correlated with Chl-a, CRDs, and Chl_T. Trehalose

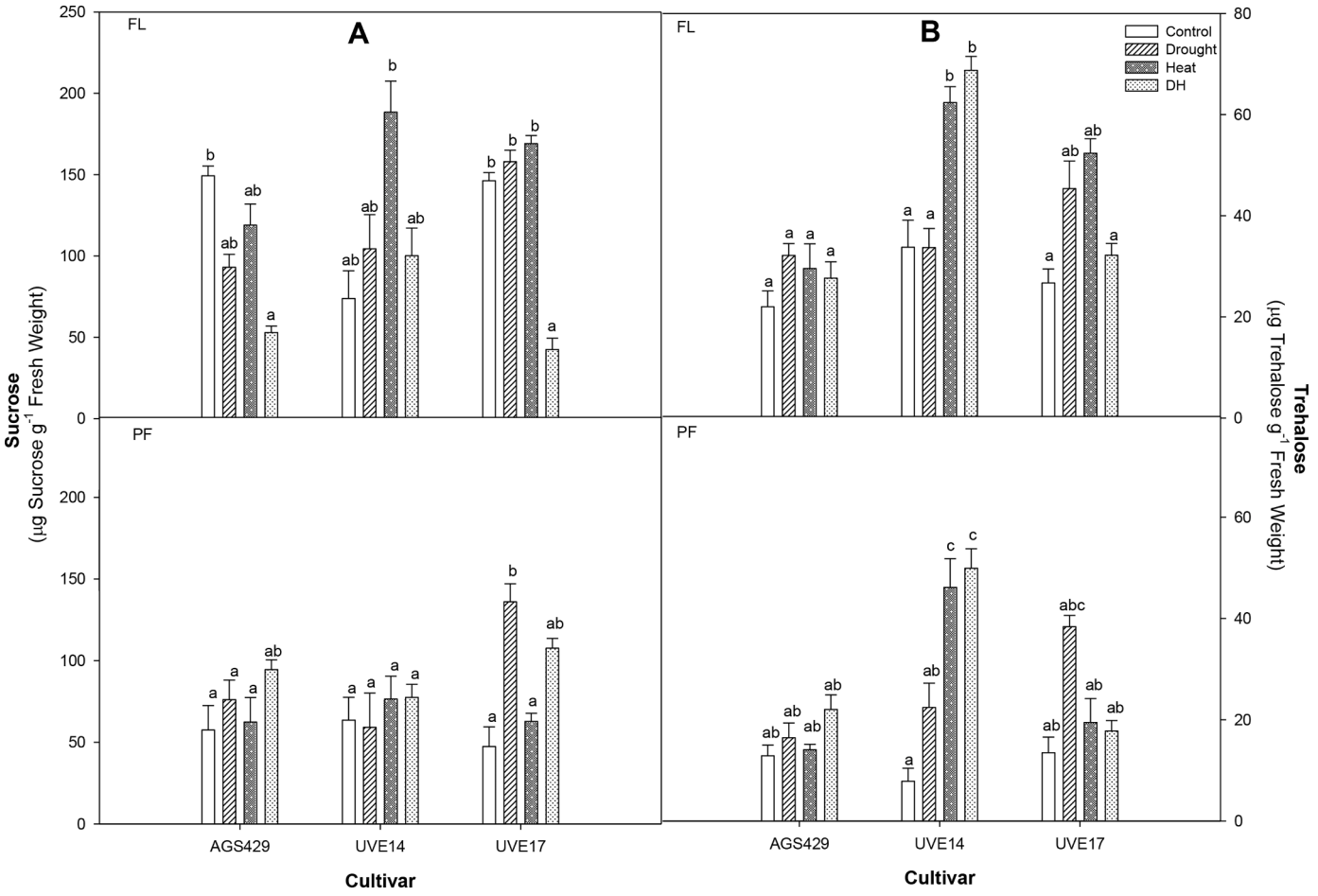

Fig. 3. Sucrose (A) and trehalose (B) content of three edamame cultivars under drought, heat, combined drought and heat (DH) stress at flowering (FL) and pod filling (PF) stages. Alphabets represent similarities or differences in sucrose or trehalose content between treatments and cultivars. Values represent means ±SD (n = 3). Control = 100% WHC + 25/18 °C, Drought = 30% WHC + 25/18 °C, Heat = 100% WHC + 35/27 °C, DH = 30% WHC + 35/27 °C. AGS429 = High yielding with moderate yield reduction under drought stress, UVE14 = low yielding with low yield reduction under drought stress, UVE17 = low yielding with high yield reduction under drought stress.

negatively correlated with δ(R_0) (Table 5). At the pod filling stage (Table 6), Fv/Fm positively correlated with sucrose, which had a strong negative correlation with CRDs. The ET_0/RC had a strong positive correlation with glucose ($P < 0.05$) while starch negatively correlated with trehalose (Table 6).

Discussion

Photosynthesis and carbohydrate metabolism, critical physiological traits influencing yield in edamame, are adversely impacted by drought stress in sensitive cultivars, compared to their tolerant counterparts (Hlahla et al., 2022). However, due to unpredictable weather changes caused by climate change, there is a need to study the physiological and biochemical responses of the edamame under combined drought and heat (DH) stress, especially in the South African context, where edamame has recently been introduced. Kalaji et al. (2018) demonstrated that DH stress reduced the rate of photosynthesis due to chloroplast damage. Other studies argued that photosynthetic pigments' concentrations are excellent indicators of plant performance under different environmental stresses (Raja et al., 2020). The DH stress can potentially impede chlorophyll production or accelerate its breakdown (Nankishore and Ferrel, 2016).

In this study, DH stress led to no degradation of the photosynthetic pigments (Chl-a, Chl-b, CRD, and total chlorophyll) at flowering growth stage in AGS42 compared to heat treatment, which led to a significant degradation of chl-b and CRDs leading to low total chlorophyll. In line with our findings, Dubberstein et al. (2020) observed metabolic and structural damages in *Coffea arabica* which was caused by the heat treatment alone. The high chlorophylls and CRDs in AGS429 under DH resulted in high NDVI (a measure of leaf greenness) and enhanced photosynthesis efficiency, suggesting that AGS429 used photosynthesis pigments protection as a mechanism for survival under DH stress. Ram et al. (2017) further showed that tolerance to DH stress in bread wheat (*Triticum aestivum* L.) is associated with increased NDVI. Similarly, the CRD content was stable in AGS429 at both growth stages under DH stress. The increased or stable CRD levels are associated with tolerance to drought, heat, or DH stress by protecting chlorophyll, whose conjugated double bonds are more exposed to rapid oxidative degradation (Collini, 2019; Mordi et al., 2020; Raja et al., 2020). Also, CRDs dissipate excitation energy of singlet state chlorophylls as heat and quench triplet state chlorophylls in antenna complexes of photosystem II to prevent photodamage (Kato and Shinomura, 2020). A positive correlation between CRDs and chlorophyll levels during flowering in AGS429 shows that these pigments have a strong dependency on one another during DH stress. Pramanik and Bera (2013) argued that CRDs protected the chloroplasts, leading to enhanced photochemical reactions, carbohydrate synthesis and improved yield under DH stress. Interestingly, the reduced CRD concentration in the UVE14 and UVE17 exposed to combined stress led to total chlorophyll content reduction, demonstrating a strong relationship between the two pigments. While AGS429 had stable

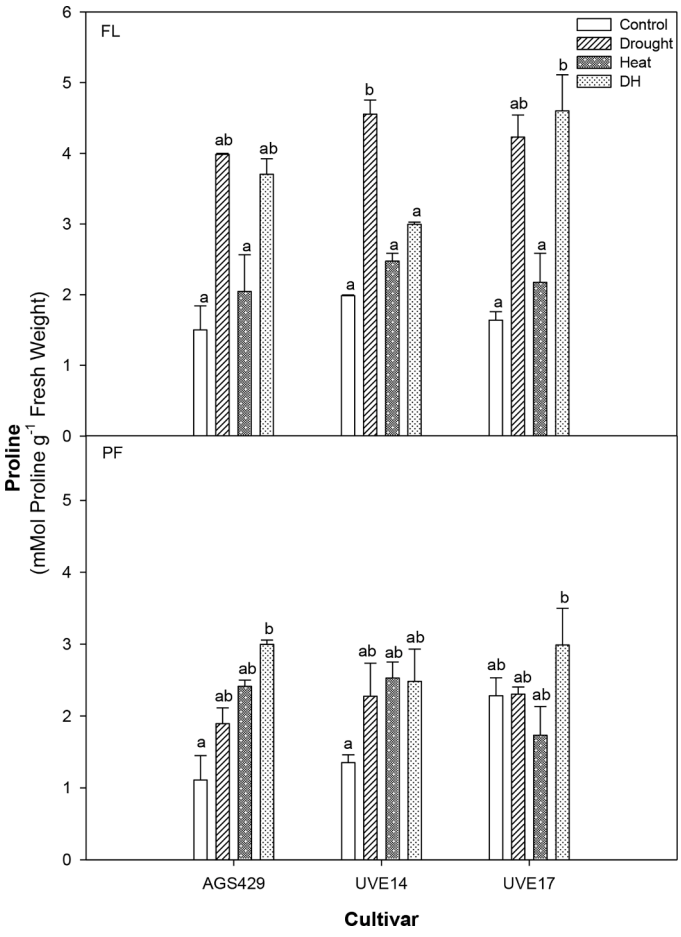

Fig. 4. Proline content of three edamame cultivars under drought, heat, combined drought and heat (DH) stress at flowering (FL) and pod filling (PF) stages. Alphabets represent similarities or differences in proline content between treatments and cultivars. Values represent means ±SD ($n = 3$). Control = 100% WHC + 25/18 °C, Drought = 30% WHC + 25/18 °C, Heat = 100% WHC + 35/27 °C, DH = 30% WHC + 35/27 °C. AGS429 = High yielding with moderate yield reduction under drought stress, UVE14 = low yielding with low yield reduction under drought stress, UVE17 = low yielding with high yield reduction under drought stress.

photosynthetic pigments at both growth stages under DH stress, UVE14 and UVE17 had improved pigments at pod filling. Thus, increased CRD content at the pod filling stage in these two cultivars under combined stress was an important adaptability strategy.

Combining destructive methods (including pigment analysis) and non-destructive photosynthetic indices emerge as a crucial approach for determining photosynthetic efficiency and photosystem performance in plants subjected to abiotic stresses such as drought, heat, and DH stress. It is worth noting that the current study addresses the photosynthetic efficiency and photosystems' performance in the three cultivars under DH stress. The PRI is a sensitive reflectance index used to detect rapid changes in the process of photosynthesis under different environmental stresses (Sukhova et al., 2022; Song et al., 2013). The PRI indicated the different carotenoids/chlorophyll (CRDs/Chl) ratios over the long term and xanthophylls de-epoxidation over the short term (Filella et al., 2009; Maoka, 2020). At pod filling, the PRI in AGS429 and UVE14 was not affected compared to the flowering stage where AGS429 also had a low PRI under DH stress, indicating an enhanced de-epoxidation of xanthophylls occurred (specifically zeaxanthin accumulation), as well as a CRD/Chl ratio increase (Havaux et al., 2007; Yudina et al., 2020). This increase in zeaxanthin concentration protected thylakoid and membrane lipids from degradation in the chloroplasts and enhanced the rate of photosynthesis in AGS429 and UVE14 at pod filling. In contrast, the UVE17 significantly reduced the PRI values (at both growth stages) upon DH stress compared to the control, drought, and heat alone. This finding validates the importance of the pigments' protection during drought, heat, and DH stress. A decrease in PRI was also observed in peas (*Pisum sativum*), wheat and mustard (*Sinapis brassicaceae*) under DH stress and was associated with reduced photosynthetic capacity (De Cannière et al., 2022; Sukhova et al., 2022).

Chlorophyll *a* fluorescence has been increasingly used as a sensitive and convenient indicator of plant response to environmental stress (Raja et al., 2020; Zhu et al., 2021). The high ABS/RC, DI_0/RC, and TR_0/RC in UVE17 because of DH (compared to the control and drought treatment) was due to deactivated or silenced reaction centres (RCs) (Mihaljević et al., 2021), which explains highest reduction in $\psi(E_0)$, Fv/Fm and PI_{ABS}. According to the results, heat treatment contributed the most to the RCs deactivation under DH because heat alone led to highest, DI_0/RC, indicating RCs damage due to heat stress in UVE17. The RCs deactivation under heat and combined DH could inhibit electron flow at PSI acceptor side, resulting in ferredoxin-$NADP^+$ reductase inactivation and poor NADPH production (Mihaljević et al., 2021). Moreover, AGS429 and UVE14 had higher $\psi(E_0)$ under DH compared to other treatments indicating normal electron flow and NADPH synthesis as shown by the least TR_0/RC in AGS429 under DH, suggesting that this cultivar had intact and active RCs. In agreement with Havaux et al. (2007), our findings suggest that the high PRI observed earlier stabilised the thylakoid membranes and enhanced RCs function in AGS429 and UVE14. Increases in ABS/RC and TR_0/RC leading to a significant PI_{ABS} decrease were observed in drought sensitive potato (*Solanum tuberosum*) (Boguszewska-Mańkowska et al., 2018) and apple (*Malus domestica* Borkh) (Mihaljević et al., 2021). At pod filling, the performance of PSII improved in all plants under all treatments compared to flowering. Similarly, the photosynthetic performance of mung bean (*Vigna radiata* L.) was altered at different points of the electron transport system (Kumar et al., 2020). Changes in electron fluxes were also observed in wheat, Chinese ash (*Fraxinus chinensis*), and China doll (*Radermachera sinica*) under combined DH stress (Zhu et al., 2021).

Since edamame is a water demanding crop (Shaheen et al., 2016), its adaptation can also be attributed to water preservation. Stomata closes to preserve water under drought conditions and opens to allow leaf evaporative cooling when exposed to high temperatures. The stomatal conductance (g_s) was significantly reduced in UVE17 at flowering, which could increase flower abortion and yield reduction (Pang et al., 2017). In contrast, under DH stress conditions, both AGS429 and UVE14 did not exhibit a significant reduction in g_s at flowering, suggesting improved production of the soluble carbohydrates. This is notable because high g_s is associated with plant survival under heat stress, indicating a favourable response to DH stress in these cultivars (Jiang and Huang, 2000). These findings agree with the previous studies showing a decrease in g_s under DH stress for tomato (*Solanum lycopersicum* L.), old man banksia (*Banksia serrata*), Queensland maple (*Flindersia brayleyana*), Golden Myrtle (*Xanthostemon Chrysanthus*), Kentucky Bluegrass (*Poa pratensis* L.) and cotton (*Gossypium barbadense* L) (Jiang and Huang, 2000; Hejnák et al., 2015; Raja et al., 2020; Marchin et al., 2022). Therefore, across the cultivars, for all photosynthetic pigments and g_s under DH, UVE17 was the most negatively affected, with AGS429 performing the best followed by UVE14.

Soluble carbohydrates concentrations are indicators of photosynthetic capacity and are associated with energy-producing metabolic processes or osmotic adjustment in plants under abiotic stress. For instance, starch and sucrose, the primary products of photosynthesis, are hydrolysed to form glucose under abiotic stress conditions in plants (Du et al., 2020). Our findings show that starch and glucose content decrease under DH stress in UVE17 could be associated with the observed reduced photochemical reactions and CO_2 fixation at both growth stages. These observations were supported by a huge reduction in stomatal conductance, indicating that starch and glucose were reduced due to lower

Table 5
The relationship between the photosynthesis efficiency parameters and various metabolites at flowering stage in edamame under combined drought and heat (30% WHC, 35 °C) stress.

	Fv/Fm	ABS/RC	DIo/RC	TRo/RC	ETo/RC	REo/RC	Ψ(Eo)	$\delta(Ro)$	PI_{ABS}	PI_{Total}	Chl-a	Chl-b	CRD	Chl_T	g_s	NDVI	PRI	starch	Glu	Suc	Pro	Treh
ABS/RC	−0,60	–																				
DIo/RC	−0,60	1,0***	–																			
TRo/RC	−0,51	0,94***	0,93***	–																		
ETo/RC	−0,45	0,81**	0,79**	0,94***	–																	
REo/RC	−0,51	0,89***	0,88**	0,98***	0,99***	–																
Ψ(Eo)	−0,79**	0,06	0,07	−0,01	0,09	0,07	–															
$\delta(R_0)$	−0,51	0,46	0,47	0,26	0,08	0,21	0,23	–														
PI_{ABS}	0,61	−0,34	−0,34	−0,35	−0,25	−0,32	−0,32	−0,44	–													
PI_{Total}	0,40	−0,39	−0,40	−0,21	−0,16	−0,24	−0,33	−0,64	0,32	–												
Chl-a	−0,43	0,26	0,26	0,29	0,12	0,18	0,09	0,55	−0,61	0,04	–											
Chl-b	−0,41	−0,12	−0,12	−0,11	−0,12	−0,11	0,45	0,24	−0,55	−0,25	0,50	–										
CRD	−0,51	0,14	0,14	0,14	0,03	0,08	0,33	0,52	−0,66	−0,21	0,80**	0,89***	–									
Chl_T	−0,48	0,04	0,04	0,06	−0,02	0,01	0,35	0,42	−0,66	−0,15	0,81**	0,91***	0,98***	–								
g_s	0,51	−0,26	−0,25	−0,37	−0,36	−0,33	−0,33	0,17	0,30	−0,30	−0,49	−0,53	−0,56	−0,59	–							
NDVI	0,16	0,37	0,37	0,37	0,28	0,32	−0,47	0,24	0,21	0,05	0,15	−0,01	0,21	0,06	−0,07	–						
PRI	0,68*	−0,35	−0,37	−0,14	0,00	−0,09	−0,54	−0,47	0,22	0,08	−0,25	−0,15	−0,32	−0,22	0,26	−0,13	–					
starch	0,25	−0,24	−0,24	−0,23	−0,29	−0,28	−0,28	−0,24	−0,16	0,12	0,00	0,52	0,38	0,35	−0,24	0,35	0,05	–				
Glu	0,33	−0,36	−0,35	−0,40	−0,28	−0,33	0,03	−0,54	0,40	−0,09	−0,83**	−0,43	−0,76*	−0,68*	0,43	−0,62	0,34	−0,15	–			
Suc	−0,25	0,70*	0,68*	0,87**	0,92***	0,90***	−0,11	0,16	−0,26	−0,22	0,22	−0,09	0,08	0,04	−0,19	0,31	0,28	−0,29	−0,34	–		
Pro	0,24	−0,47	−0,48	−0,37	−0,35	−0,37	−0,12	0,08	−0,06	0,49	0,41	−0,10	0,07	0,12	0,19	−0,18	0,10	−0,28	−0,29	−0,18	–	
Treh	0,35	0,09	0,07	0,27	0,38	0,29	−0,38	−0,70*	0,37	0,26	−0,34	−0,20	−0,36	−0,30	−0,31	0,09	0,61	0,14	0,32	0,37	−0,45	–

* $P \leq 0.05$,.
** $P \leq 0.01$,.
*** $P \leq 0.001$, Fv/Fm = Ratio of variable fluorescence to maximal fluorescence of PSll, PI_{ABS} = Performance index of photosystem I (PSl) and photosystem II (PSll), PI_{Total} = Total performance index of PSl and PSll, ABS/RC = Energy absorbed per reaction centre (RC), DI_0/RC = Energy dissipated as heat per RC, TR_0/RC = trapped energy per RC, ET_0/RC = the flux of electrons transferred from quinone (Q_A^-) to plastoquinone (PQ) per active PSll per RC, RE_0/RC = electron flux reducing end electron acceptors at PSl acceptor side per RC, $\psi(E_0)$ = efficiency that an electron moves further than Q_A^-, $\delta(R_0)$ = probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSl acceptor side, PRI = Photochemical reflectance index, g_s = stomatal conductance, Chl-*a* = chlorophyll *a*, Chl-*b* = chlorophyll *b*, CRD = carotenoids, Chl_T = Total chlorophyll, NDVI = normalised difference vegetation index, Treh = Trehalose, Glu = Glucose, Suc = Sucrose, Pro = Proline. AGS429 = High yielding with moderate yield reduction under drought stress, UVE14 = low yielding with low yield reduction under drought stress, UVE17 = low yielding with high yield reduction under drought stress.

Table 6
The relationship between the photosynthesis efficiency parameters and various metabolites at pod filling stage in edamame under combined drought and heat (30% WHC, 35 °C) stress.

	Fv/Fm	ABS/RC	DIo/RC	TRo/RC	ETo/RC	REo/RC	Ψ(Eo)	$\delta(Ro)$	PI_{ABS}	PI_{Total}	Chl-a	Chl-b	CRD	Chl_T	g_s	NDVI	PRI	starch	Glu	Suc	Pro	Treh
Fv/Fm	–																					
ABS/RC	−0,07	–																				
DIo/RC	−0,44	0,93***	–																			
TRo/RC	0,03	0,99***	0,89**	–																		
ETo/RC	0,41	0,73*	0,50	0,7706*	–																	
REo/RC	−0,49	−0,16	0,06	−0,21	−0,11	–																
Ψ(Eo)	0,49	−0,75*	−0,86	−0,70*	−0,10	0,16	–															
$\delta(Ro)$	−0,60	−0,49	−0,20	−0,55	−0,60	0,86**	0,16	–														
PI_{ABS}	0,57	−0,79**	−0,92	−0,74*	−0,27	0,04	0,93***	0,15	–													
PI_{Total}	0,12	−0,87	−0,81	−0,86	−0,54	0,48	0,78**	0,65	0,85**	–												
Chl-a	−0,58	0,12	0,34	0,06	−0,43	0,03	−0,60	0,25	−0,48	−0,21	–											
Chl-b	−0,03	0,28	0,26	0,28	0,20	−0,40	−0,25	−0,44	−0,35	−0,54	0,00	–										
CRD	−0,54	0,02	0,20	−0,03	−0,54	−0,12	−0,57	0,20	−0,49	−0,26	0,41	0,10	–									
Chl_T	−0,53	0,23	0,42	0,18	−0,30	−0,14	−0,65	0,04	−0,58	−0,43	0,90***	0,43	0,41	–								
gs	0,30	0,32	0,17	0,35	0,28	0,00	−0,23	−0,10	−0,21	−0,23	−0,23	0,03	0,28	−0,20	–							
NDVI	0,55	−0,29	−0,47	−0,24	0,00	−0,36	0,37	−0,29	0,43	0,18	−0,27	0,25	−0,37	−0,14	0,11	–						
PRI	0,31	0,74*	0,53	0,77**	0,80**	−0,29	−0,35	−0,62	−0,48	−0,71*	−0,33	0,43	−0,12	−0,11	0,60	0,26	–					
starch	−0,01	0,15	0,14	0,16	0,40	0,19	0,17	−0,05	0,00	−0,03	−0,02	−0,42	−0,40	−0,20	−0,36	−0,52	−0,11	–				
Glu	0,30	0,55	0,39	0,58	0,71*	−0,04	−0,13	−0,39	−0,21	−0,38	0,15	−0,03	−0,54	0,12	0,06	−0,12	0,38	0,63	–			
Suc	0,77*	0,06	−0,22	0,13	0,50	−0,11	0,42	−0,34	0,48	0,20	−0,51	−0,10	−0,80**	−0,50	0,15	0,64	0,35	−0,06	0,33	–		
Pro	0,59	−0,21	−0,42	−0,15	0,07	−0,47	0,34	−0,42	0,34	0,00	−0,40	0,62	−0,07	−0,09	0,31	0,52	0,25	−0,46	−0,11	0,24	–	
Treh	−0,41	−0,01	0,15	−0,05	0,10	0,50	0,14	0,34	−0,06	0,12	0,12	−0,35	−0,19	−0,04	−0,49	−0,72*	−0,38	−0,87**	0,34	−0,37	−0,54	–

* $P \leq 0.05$,.
** $P \leq 0.01$,.
*** $P \leq 0.001$, Fv/Fm = Ratio of variable fluorescence to maximal fluorescence of PSII, PI_{ABS} = Performance index of photosystem I (PSI) and photosystem II (PSII), PI_{Total} = Total performance index of PSI and PSII, ABS/RC = Energy absorbed per reaction centre (RC), DI_0/RC = Energy dissipated as heat per RC, TR_0/RC = trapped energy per RC, ET_0/RC = the flux of electrons transferred from quinone (Q_A^-) to plastoquinone (PQ) per active PSII per RC, RE_0/RC = electron flux reducing end electron acceptors at PSI acceptor side per RC, $\psi(E_0)$ = efficiency that an electron moves further than Q_A^-, $\delta(R_0)$ = probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side, PRI = Photochemical reflectance index, g_s = stomatal conductance, Chl-*a* = chlorophyll *a*, Chl-*b* = chlorophyll *b*, CRD = carotenoids, Chl_T = Total chlorophyll, NDVI = normalised difference vegetation index, Treh = Trehalose, Glu = Glucose, Suc = Sucrose, Pro = Proline.

photosynthesis efficiency at both growth stages. In contrast, AGS429 and UVE14 displayed a lower starch concentration but their glucose concentration was higher than that of UVE17. These observations suggest that the starch hydrolysis was linked to increased glucose concentration in AGS429 and UVE14 samples exposed to DH stress at the pod-filling stage. Sehgal et al. (2017) and El Habti et al. (2020) observed increased starch hydrolysis due to high glucose demand under DH stress in wheat. In addition, starch was hydrolysed to supply glucose in soybeans under drought stress (Du et al., 2020), which is argued to be essential for seed filling and maintaining cell turgor (El Habti et al., 2020).

Sucrose is another essential carbohydrate that regulates hexose-sucrose balance and seed filling processes (Awasthi et al., 2014). In AGS429, the low sucrose content at flowering under DH stress was due to its hydrolysis to provide glucose and fructose. In contrast, we propose that the low sucrose content in UVE17 was due to reduced photosynthetic efficiency influenced by reduced stomatal conductance and reaction centre activities. Furthermore, DH stress led to the production of sucrose at pod filling in all the cultivars. Similarly, sucrose accumulation during DH stress was reported in wheat samples (Sehgal et al., 2017). In contrast, Awasthi et al. (2014) recorded reduced sucrose content at pod filling under DH stress in chickpeas. Like sucrose, trehalose is an important non-reducing disaccharide, which is known to protect biological structures under drought or heat conditions (Kosar et al., 2019; Di Gioacchino et al., 2021). At both growth stages, heat treatment and DH stress induced high trehalose accumulation in UVE14 and this accumulation was highest under combined stress. The drought tolerant cultivar, AGS429 also accumulated high trehalose content at pod filling under DH stress. The negative correlation of trehalose with starch under DH stress at pod filling indicated that starch hydrolysis produced precursor molecules for trehalose synthesis, accumulating in AGS429 and UVE14. Hlahla et al. (2022) showed that trehalose accumulated in AGS429 and UVE14 exposed to drought stress, and it played an important role in osmotic adjustment. In addition to its osmotic adjustment function, trehalose has antioxidant activity that helps stabilise proteins and membranes, promoting electron transport and protecting photosystems (Luo et al., 2008, 2021). In this study, the degradation of starch to form glucose and trehalose precursors in AGS429 and UVE14 serves as DH stress survival strategy that could adjusting leaf turgor pressure and stabilise biological structures. However, sucrose was produced solely to support plant growth and development across all cultivars.

Proline accumulation is known to increase drought tolerance in tobacco (*Nicotiana tabacum* L.), watermelon (*Citrullus lanatus*) and edamame through maintenance of osmotic balance (Cvikrová et al., 2013; Moloi and van der Merwe, 2021; Wang et al., 2022). Some studies associate proline with antioxidant protective roles under heat stress (Tonhati et al., 2020). The accumulation of proline under drought stress may be driven by reduced osmotic balance, as drought alone increased proline levels in all cultivars during flowering. Although proline did not accumulate under heat treatment, its production was increased significantly under DH stress in AGS429 and UVE17. At pod-filling, proline content was low in UVE17 under DH stress while it accumulated to high levels in AGS429 and UVE14. The elevated proline content observed in these cultivars during pod filling under DH stress may enhance their osmotic potential (Wang et al., 2022), which potentially aids in stabilising proteins and plasma membranes. However, in this case, proline may not be one of the defence strategies implemented for DH stress tolerance in edamame because it was also increased in UVE17.

Conclusions

The physiological and biochemical responses indicated that UVE17 exhibited susceptibility to combined drought and heat (DH) stress through reduced photosynthetic efficiency (lower Chl-b, CRD, total chlorophyll content, Fv/Fm, ψ(E0), PI_{ABS}, g_s, PRI, and high DI_0/RC), along with reduced CO_2 assimilation (lower g_s), during the flowering stage, which reduced accumulation of carbohydrate metabolites used for DH stress resilience. Conversely, increased carotenoid accumulation in AGS429 showed that tolerance to DH stress in this cultivar is achieved through preservation of the light-harvesting pigments (Chl-a, Chl-b, total chlorophyll, and intact reaction centres), which resulted in high photochemical reactions. In addition, this cultivar increased its CO_2 fixing capacity by reducing stomatal closure, which led to elevated glucose and sucrose. It also increased starch hydrolysis with high accumulation of trehalose, thereby promoting osmotic adjustment. Similarly, UVE14 responded to DH stress by increasing its photosynthesis efficiency, which led to higher accumulation of carbohydrates. We established for the first time that increased photosynthesis efficiency and carbohydrate metabolites under DH stress are crucial adaptation features in edamame. Given these physiological and biochemical responses, AGS429 and UVE14 are promising cultivars for breeding programs aimed at enhancing DH stress resilience, thereby contributing to food security.

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Grammarly and ChatGPT in order to improve readability of parts of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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CRediT authorship contribution statement

Jeremiah M. Hlahla: Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding acquisition, Formal analysis, Data curation. **Mpho S. Mafa:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization. **Rouxléne van der Merwe:** Writing – review & editing, Supervision, Software, Formal analysis. **Makoena J. Moloi:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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