Environmental and Experimental Botany 222 (2024) 105769

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Research paper

Negative synergistic effects of drought and heat during flowering and seed setting in soybean

Sadikshya Poudel [a], Ranadheer Reddy Vennam [a], Lekshmy V. Sankarapillai [a], Jinbao Liu [b], K. Raja Reddy [a], Nuwan K. Wijewardane [c], M. Shahid Mukhtar [b], Raju Bheemanahalli [a,*]

[a] *Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS, USA*
[b] *Department of Biology, University of Alabama-Birmingham, Birmingham, AL, USA*
[c] *Department of Agricultural & Biological Engineering, Mississippi State University, Mississippi State, MS, USA*

ARTICLE INFO

Keywords:
Interactive stress
Physiology
Vegetative indices
Seed quality
Gene expression

ABSTRACT

Rising intense heat waves combined with lower precipitations are the new norms of current global scenarios. These altered climatic conditions negatively impact soybean yield potential and quality. Ten soybean cultivars were subjected to four different growing conditions: control, drought, heat, and combined drought and heat to understand the physiological, yield, quality and molecular changes. Stomatal conductance was reduced by 62% and 10% under drought and heat, respectively. This reduction was further exacerbated to 93% when exposed to combined stress. The highest canopy temperature was recorded at +8 °C with combined stress treatment, whereas drought and heat exhibited +2 °C and +5.4 °C, respectively. Furthermore, combined stress displayed a more pronounced negative impact on greenness-associated vegetative index; the gene expression analysis further corroborated these findings. Each degree Celsius increase in temperature during flowering and seed-filling reduced seed weight by approximately 7% and 4% with and without drought, respectively. The seed protein increased under drought, whereas the oil showed a converse trend under drought and combined stresses. Most physiological and yield traits showed no significant correlations between control and individual or combined stress. This suggests that selecting crops for combined stress tolerance may not be appropriate based on nonstress or individual stress performance. Thus, incorporating combined stress-resilient traits into elite soybean cultivars could significantly boost soybean production under hot and dry climatic conditions.

1. Introduction

Soybean (*Glycine max* [L.] Merr.) is a leading oilseed crop grown in various climatic conditions (Li et al., 2017; Zhu et al., 2020). After corn, soybean is the most widely planted crop in the United States, accounting for 32% of the total cultivated land (Annual Soy Stats Results, 2021). Although the USA is the second largest soybean producer, >90% of its soybean is produced under rainfed conditions. About 51% of soybean-growing regions in the US are exposed to drought (USDA Drought Monitor, 2023), and 80% of these areas are affected by heat stress (USDA ERS, 2023). Approximately 75% of the soybean acreage in Mississippi is rainfed (Zhang et al., 2016), with no supplementary irrigation. Based on increasing temperatures and extended periods of insufficient rains in recent years, a yield decline of up to 92% by the year 2050 has been projected (Yu et al., 2021a). Many studies have shown that the impact of combined stress during the reproductive stage was greater than in the early vegetative stage in crops (Jumrani and Bhatia, 2018). Most southern US soybean-growing areas are exposed to these climatic conditions during critical growth stages (Poudel et al., 2023a). With an increasing frequency of heatwaves and prolonged drought spells during the reproductive seed-fill stages, soybean production is predicted to face significant yield and quality losses.

Exposure of soybean cultivars to drought stress during the reproductive and seed-filling stages decreased seed number and weight (Poudel et al., 2023b). These decreases were associated with reduced stomatal conductance and increased canopy temperature under drought. Whereas under heat stress, stomatal conductance and transpiration were increased or on par with control, but seed number (4.2%) and seed weight (5%) reduced per degree Celsius increase in temperature over 32 °C (Poudel et al., 2023a). For every 1 °C increase above average temperature, soybean yields are expected to decline by 2.4% (Hatfield et al., 2011; Alsajri et al., 2022). The decrease in leaf size and

---

* Corresponding author.
E-mail address: rajubr@pss.msstate.edu (R. Bheemanahalli).

https://doi.org/10.1016/j.envexpbot.2024.105769
Received 17 February 2024; Received in revised form 5 April 2024; Accepted 6 April 2024

cell membrane stability causes a reduction in CO_2 assimilation due to an increase in canopy temperature (Onat et al., 2017). Under stress conditions, chloroplasts experience oxidative damage (Vennam et al., 2023a), which primarily affects the photosynthetic process and accelerates leaf senescence by activating metabolic changes at the source (leaf) and sink (seed) (Wang et al., 2008; Hatfield et al., 2011). However, soybean genotypes respond differently to individual heat or drought stress, with some genotypes exhibiting significant plasticity (Poudel et al., 2023a). Small-seeded soybean genotypes were less sensitive to heat stress than large-seeded soybean (Puteh et al., 2013). However, little is known about soybean's genetic variability to combined stress during the reproductive stage.

The intricate interplay of combined stressors threatens agriculture production. These interactive stressors elicit unique physiological, yield, and metabolic responses in plants, distinguishing them from responses triggered by individual stressors (Mittler, 2006; Prasch and Sonnewald, 2013; Cohen et al., 2021b). Previous studies demonstrated that the combined stress impact is much more significant and complex depending on crops' growth stages (Schauberger et al., 2017; Matiu et al., 2017). Studies on other crops reveal that combined drought and heat stress have a far greater impact on growth, reproduction, and grain-filling processes than the single stress (Bheemanahalli et al., 2022a). Stressors that disrupt the crucial developmental stages of plants, such as pollination, fertilization, and seed formation, cannot be mitigated in later growth stages (Krishnan et al., 2020; Poudel et al., 2023b). Notably, the interaction of drought and heat around flowering disrupts reproductive success and physiological-biochemical functions associated with seed-filling. On the other hand, plants reduce carboxylation and produce less sucrose and starch under stressors. This can lead to decreased seed size and weight due to the deactivation of biosynthetic enzymes and less availability of sugars to convert into storage food (Prasad et al., 2017; Zinta et al., 2018). Additionally, transcriptome analyses conducted on tobacco leaves under combined drought and heat reveal the suppression of photosynthetic gene expression and the induction of genes associated with glycolysis and the pentose phosphate pathway (Rizhsky et al., 2002). This molecular crosstalk reduced plant photosynthetic capacity and shortened seed-filling duration, potentially influencing seed yield and quality. Seed quality is a critical component of marketability in soybean production, where genetic factors and the growing environment influence the composition of seed protein, oil, and fatty acids. Although soybean-growing regions are prone to drought and heat stress, there is limited information on these combined stresses (Jumrani and Bhatia, 2018; Ergo et al., 2018; Cohen et al., 2021b).

In addition, unique plant responses to combined stress have been identified to be governed by complex and distinct regulatory mechanisms (Zhang and Sonnewald, 2017; Cohen et al., 2021b; Sinha et al., 2023). A recent phenotypic-transcriptomic analysis of soybean plants subjected to individual and combined stressors revealed that different tissues displayed unique transcriptomic responses (Sinha et al., 2023). However, limited studies ascertained the impact of these synergistic stressors on physiology, spectral properties, yield, and quality traits in soybean. Further, it is crucial to understand how well high-yielding soybean cultivars handle these challenges, as they may exhibit more varied responses. This study investigated the effects of individual and combined stressors on soybean, focusing on three key areas: (i) plant physiology and spectral properties, (ii) yield and seed quality, and (iii) the relationships of these traits among stress conditions.

2. Materials and methods

2.1. Plant materials

This study used ten soybean cultivars belonging to maturity groups IV and V recommended for the Midsouth US region. Among these cultivars, eight were commercially available for growers, and two (R15–2422 and R01–416F) were advanced breeding lines (Supplementary Table 1). R15–2422 was derived from crossing high-yielding conventional maturity group IV parents resistant to Cercospora leaf blight. R01–416F was the registered germplasm developed by crossing between Jackson and KS4896 to improve yield and nitrogen fixation under drought stress (Chen et al., 2007).

2.2. Crop husbandry

The experiment was conducted at Rodney Foil Plant Science Research Center of Mississippi State University, Mississippi, USA (33°28' N, 88°47' W) using a greenhouse facility. Four seeds per cultivar were sown in a 13.5 L pot filled with farm soil. A 4-gram of slow-release fertilizer Osmocote (N:P:K - 14:14:14, Hummert International) was added to the pot after sowing and top-dressed before flowering. A systemic insecticide Marathon 1% G (Imidacloprid, OHP, Mainland, PA) was applied to each pot (4 g) after seedling emergence to avoid infestation of sucking pests. After emergence, each pot was thinned down to a single plant. A total of 320 plants (ten cultivars × eight replicates × four treatments) were grown in a greenhouse under ideal conditions (32/24 °C day/night temperatures) for 50 days (until first flowering; R1 stage, Fehr and Caviness, 1977). Plants were regularly monitored and watered through pre-programmed time-based drip irrigation to maintain moisture above 0.15 m^3 m^{-3} volumetric water content (VWC).

2.3. Stress treatment conditions

At full bloom (R2 stage; Fehr and Caviness, 1977), the plants were divided into four groups and moved to two greenhouses. One hundred and sixty pots were maintained in a greenhouse with 32 °C day temperature (current growing climate). Among them, 80 pots were provided with 100% irrigation (0.15 m^3 m^{-3} VWC; control, CNT), and the remaining with 50% irrigation of the control (drought stress, DS). The remaining 160 pots were maintained in another greenhouse with 38 °C day temperature (future or warmer growing climate). Among them, 80 pots were provided with 100% irrigation (heat stress, HS), and the remaining with 50% irrigation (combined drought and heat, DS+HS). A common nighttime temperature (24 °C) was maintained in both the greenhouses. The thermostat, cooling pad, and ventail flaps were programmed to maintain the set temperatures inside the greenhouse (Bheemanahalli et al., 2022a). HOBO data loggers (Onset Computer Corporation, Bourne, MA 02532, USA) were installed above the crop canopy for each treatment condition to monitor the microclimatic greenhouse conditions (temperature and relative humidity) throughout the experiment. Forty soil moisture probes (Model EM5b Soil Moisture, Decagon Devices, Inc., Pullman, WA, USA) were installed to monitor volumetric water content (VWC) at 15 cm depth across all the treatments at 15-min intervals. Stress was imposed for 30 days from the R2 (full bloom stage; Fehr and Caviness, 1977) to the R6 (full seed stage; Fehr and Caviness, 1977) stage. After 30 days of stress, all plants were grown under control conditions (32 °C day temperature and 100% irrigation) and maintained until maturity.

2.4. Data collection

2.4.1. Leaf pigments and physiological parameters

Leaf pigments such as chlorophyll and anthocyanin indexes were recorded using a handheld Dualex® Scientific instrument (Force A DX16641, Paris, France). The physiological parameters (stomatal conductance and transpiration) were measured using a portable handheld LI600 porometer system integrated with a fluorometer (LI-COR Biosciences, Lincoln, USA) across the treatments. These parameters were recorded after every two-day interval throughout the stress period. At 14 days of stress, the photosynthesis measurement was made under an artificial irradiance of 1500 μmol (photons) m^{-2} s^{-1}, 420 μmol CO_2 mol^{-1}, and a constant flow rate of 600 μmol m^{-2} using portable LICOR 6800 (LI-COR Biosciences, Lincoln, USA). All the pigments and physiological parameters were measured from the third fully expanded trifoliate leaf from the apical end. The canopy temperature was measured during solar noon using the handheld MI-2300 infrared radiometer (Apogee Instruments Inc., Logan, UT, USA).

2.4.2. Leaf spectral signatures and vegetation indices

To evaluate the impact of treatments on leaf biophysical properties, leaf hyperspectral data (350–2500 nm) were collected using a PSR$^+$3500 spectroradiometer (Spectral Evolution, Massachusetts, USA). Using the leaf clip accessory, measurements were taken on the third fully expanded trifoliate leaf between 10:00 h and 13:00 h . Four random replicates of each cultivar under each treatment were scanned thrice to reduce measurement noise. A white reference panelboard within the leaf clip calibrated the instrument every 30 min. Five sets of spectral bands were employed to calculate the vegetation indices (VIs) to match the proximal sensing to the commercially available MicaSense RedEdge multispectral sensor (Bheemanahalli et al., 2022a). These bands encompassed blue (centered at 475 nm/ bandwidth of 32), green (centered at 560 nm/ bandwidth of 28), red (centered at 668 nm/ bandwidth of 16), red-edge (centered at 717 nm/ bandwidth of 12), and near-infrared band (centered at 842 nm/ bandwidth of 58 nm), from which six VIs: chlorophyll index of green (CIgreen, Gitelson et al., 2003), chlorophyll index of red-edge (CIred-edge, Steele et al., 2008), chlorophyll vegetation index (CVI, Vincini et al., 2008), normalized difference red-edge index (NDRE, Thompson et al., 2019), transformed chlorophyll absorption in reflectance index (TCARI, Haboudane et al., 2002), and visible atmospherically resistant index (VARI, Gitelson et al., 2002), were derived using equations given in Supplementary Table 2.

2.4.3. Yield and quality components

The replicated plants were manually harvested at physiological maturity (R8 stage; Fehr and Caviness, 1977) to obtain the yield and yield components. The shoot and pods were separated from each plant. The pods were counted, weighed, and later threshed manually to obtain the seed weight. The number of seeds per plant was determined using a seed counter (NP5056-Model 850–2, LI-COR, Lincoln, NE, USA). The hundred seed weight was determined to estimate the impact of treatment on seed size. After collecting the yield, seed quality was assessed using a Perten DA7250 (Perten Instruments, Springfield, IL, USA). The scanning was performed using the default setting and calibrations developed by the DA7250 manufacturer for soybean seed samples (Bheemanahalli et al., 2022b).

2.4.4. RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

The leaves of all cultivars were sampled during the flowering and seed-setting stage, with four biological replicates for each treatment. Leaf samples weighing 200 mg were ground with 1 ml TRIzol using a pestle and mortar. Total RNA was isolated following the TRIzol manufacturer's instructions (Invitrogen) and quantified using a BioPhotometer Plus (Eppendorf AG, Hamburg, Germany). To remove DNA contamination, 10 ug of RNA was subjected to sequential DNase treatment using the TURBO DNA-free™ Kit (Ambion). Following the manufacturer's instructions, reverse transcription was performed using 3 ug RNA and the SuperScript IV reverse transcriptase first-strand synthesis kit (Invitrogen). PCR programs were run on an Applied Biosystems 96-Well Thermal Cycler (Eppendorf AG, Hamburg, Germany) for the DNase treatment and reverse transcription reaction. qRT-PCR was performed on an ABI 7500 Fast PCR System (ThermoFisher Scientific, Waltham, MA, USA), using the 2X PowerUp SYBR green master mix (Applied Biosystems, ThermoFisher Scientific) with the following settings: 50 °C for 2 min and 95 °C for 10 min followed by 40 cycles of 95 °C for 15 sec, 55 °C for 15 sec and 72 °C for 1 min. Gene expression analysis was carried out using three stress-responsive genes, namely GLYMA.10G23600 (drought), GLYMA.07G109100 (heat), and GLYMA.03G30040 (combined), based on homolog searches and literature (Wang et al., 2018; Xu et al., 2019). ELF1b was chosen as a reference gene for quantifying gene expression. Two technical replicates from each of the four biological replicates were used to calculate relative gene expression. The average Ct value from the technical replicates of each biological replicate was first calculated. The relative gene expression was calculated using the 2ˆ(-ΔΔCt) method, where ΔΔCt = (Ct of ELF1b - Ct of target gene) (Livak and Schmittgen, 2001). Primer sequences can be found in Supplementary Table 3.

2.5. Statistical analysis

The experimental design was a split-plot randomized complete block design, with stress treatment as the main factor and the cultivars as the subplot factor. The significance of treatment, cultivar, and their interaction for all the parameters was analyzed using the library "lmer", "lsmeans" and "agricolae" (Bates et al., 2015; Lenth, 2016; Mendiburu and Yaseen, 2020). The post hoc Fisher's Least Significant Difference (LSD) was used for mean separation, and differences were considered significant at $p< 0.05$. Data were analyzed using the statistical software R version 4.2.2 (https://www.R-project.org/, R Core). Correlation analyses were conducted to determine whether soybean cultivars exhibited unique responses to individual and combined stress treatments. The stress tolerance index (STI) was calculated for the ten soybean cultivars under drought, heat, and combined treatments for physiology (chlorophyll content, anthocyanin, stomatal conductance, transpiration, canopy temperature, photosynthesis), leaf reflectance (CI green, CI red-edge, CVI, NDRE, TCARI, VARI), yield (pod number/weight, and seed number/ weight), quality (protein, oil, linoleic acid, linolenic acid, oleic acid, sucrose) parameters, and gene expression data (GLYMA.10G23600, GLYMA.07G109100, GLYMA.03G30040) using the formula defined by Fernandez (1992).

$$Stress \quad Tolerance \quad Index = \frac{Ys \times Yc}{(Xc)^2}$$

Ys is the phenotypic mean of a given cultivar under a given stress, and Yc is the phenotypic mean of a given cultivar under control. Xc is the mean yield of all cultivars under control.

The cultivars were ranked based on the stress tolerance index value. Based on the physiology, leaf reflectance, yield, and gene expression stress tolerance index, each cultivar was assigned a score from 1 (sensitive) to 10 (tolerant). The library "ggpubr" was used to generate the bubble plot. All graphs were generated using the library "ggplot2" in R and Sigma Plot 14.5 (Systat Software, San Jose, CA, USA).

3. Results

3.1. Temperature and soil moisture content

Based on the historical and projected monthly precipitation and maximum temperature during the reproductive period in the southern US, two soybean growing environments (current and warmer growing temperatures with optimum and low precipitation) were replicated during the flowering and seed-setting stages. Ten soybean cultivars were exposed to four treatments to examine the resilience to stressors during flowering to the seed-setting stage (Fig. 1). During the treatment period, the VWC was maintained at 0.15 $m^3 \ m^{-3}$ ± 0.03 under control, 0.06 $m^3 \ m^{-3}$ ± 0.01 under drought, 0.14 $m^3 \ m^{-3}$ ± 0.03 under heat, and 0.05 $m^3 \ m^{-3}$ ± 0.01 under combined stress for 30 days (Fig. 1a). The average maximum daytime air temperature was maintained at 34.7 °C ± 1.8 under control and drought stress, while under heat and combined stress, the temperature was 8.4 °C higher than the control during the stress period (Fig. 1b).

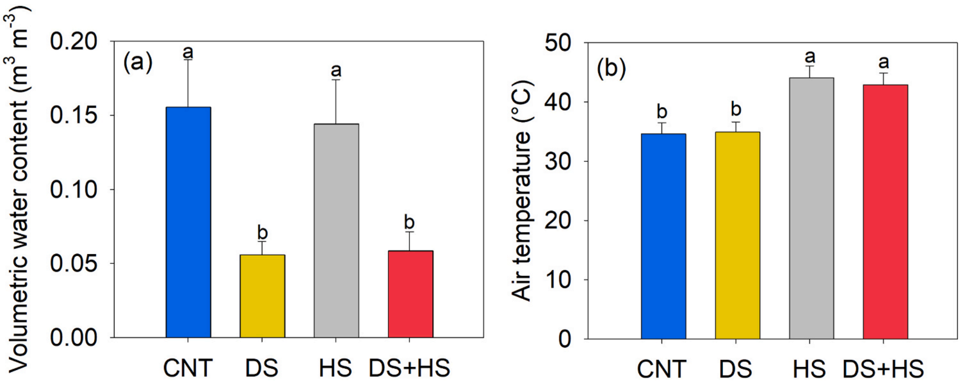

Fig. 1. Volumetric water content (a) and maximum air temperature (b) during the treatment period. The bar graphs represent the average over 30 days ± SD. CNT – control, DS – drought stress, HS – heat stress, and DS+HS – drought and heat stress. Means followed by the same letter are not significantly different at $p < 0.05$.

3.2. Pigments and physiological traits

Stress induced a significant ($p < 0.001$) effect on pigments and physiological parameters (Table 1). The cultivar × treatment interaction effect was found to be significant ($p < 0.05$) for stomatal conductance (*gs*), transpiration rate, and anthocyanin content at 14 days of stress (R4 stage-full pod) (Table 1). Under drought stress, there was an initial increase in chlorophyll content (Supplementary Fig. S1a), and it remained relatively stable under control and drought stress. However, there was a 16% and 10% reduction under heat and combined stress, respectively, compared to the control (Table 1; Fig. 2a). The cultivar DM45X61 (23% decrease) and LS5009XS (18% decrease) displayed a maximum reduction in chlorophyll content under heat and combined stress compared to the control (Fig. 2a). R15–2422 performed consistently better under drought, heat, and combined stress, with the lowest reduction in chlorophyll content compared to the control. The interactive drought and heat stress led to a substantial increase (20%) in the anthocyanin index (Fig. 2b), with R15–2422 having a maximum increase (31%) among all the cultivars, compared to the corresponding control treatment (Fig. 2b).

There was a consistent declining trend in *gs* and transpiration under drought and combined stress (Fig. 2). Drought and heat alone or combined increased canopy temperatures compared to the control. Cultivars exposed to combined stress had a higher canopy temperature (+8 °C ± 1.5) followed by heat (+5 °C ± 1.3) and drought (+2 °C ± 1.5) compared to the control (32 °C) (Fig. 2c; Table 1). It was observed that an increase in canopy temperature had a negative association with the photosynthetic rate. Compared to the control, the photosynthetic rate was reduced by 33% and 31% under drought and heat stress, respectively (Fig. 2f; Table 1). Combining both stresses caused a 50% reduction in the photosynthetic rate (Fig. 2). Different cultivars responded differently to individual and interactive stress. Specifically, the cultivar DM45X61, which had the highest *gs* and photosynthetic rate under control, maintained a comparatively higher photosynthetic rate under drought. Under combined stress, R15–2422 recorded a maximum *gs*, transpiration, and photosynthetic rate, whereas 4775E3S showed the least performance. Under combined stress, R15–2422, with high chlorophyll content and stomatal conductance, maintained the highest photosynthetic rate compared to other cultivars (Fig. 2). Despite significant reductions in stomatal conductance under drought and combined stress compared to heat, the rate of photosynthesis remained comparable under individual stresses and significantly reduced under

Table 1
Analysis of variance and mean values of the pigment, physiological, yield, and quality components of ten soybean cultivars (C) under control (CNT), drought (DS), heat (HS), and combined drought and heat (DS+HS) treatments (T).

Trait	T	C	T x C	CNT	DS	HS	DS+HS
Chlorophyll content (Chl, μg cm^{-2})	***	***	ns	33.6^{a}	34.8^{a}	28.11^{c}	29.83^{b}
Anthocyanin index (Anth)	**	***	*	0.12^{b}	0.12^{b}	0.13^{b}	0.14^{a}
Stomatal conductance (gs, mol m^{-2} s^{-1})	***	***	***	1.02^{a}	0.38^{c}	0.92^{b}	0.06^{c}
Transpiration rate (E, mmol m^{-2} s^{-1})	***	***	***	8.29^{a}	5.51^{b}	11.70^{a}	2.67^{b}
Canopy temperature (CT, °C)	***	*	ns	32.0^{d}	34.0^{c}	37.6^{b}	40.3^{a}
Photosynthetic rate (A, μmol m^{-2} s^{-1})	***	ns	ns	29.6^{a}	19.0^{b}	18.3^{b}	13.1^{c}
Pod number (PN, plant^{-1})	***	***	*	163^{a}	85.0^{c}	124.5^{b}	86.7^{c}
Pod weight (PWt., g plant^{-1})	***	*	ns	83.0^{a}	52.2^{c}	57.9^{b}	37.3^{d}
Seed number (SN, plant^{-1})	***	***	***	378.3^{a}	191.4^{c}	274.3^{b}	174.2^{d}
Seed weight (SWt., g plant^{-1})	***	***	***	55.9^{a}	36.0^{b}	36.9^{b}	23.8^{c}
Hundred seed weight (HSWt., g)	***	***	ns	14.8^{b}	18.9^{a}	13.7^{c}	13.9^{c}
Protein (% dry basis)	***	***	**	40.3^{b}	43.3^{a}	40.6^{b}	40.7^{b}
Oil (% dry basis)	***	***	***	23.2^{b}	20.5^{d}	23.8^{a}	22.8^{c}
Linoleic acid (% dry basis)	***	***	*	52.5^{a}	46.3^{b}	43.4^{c}	39.5^{d}
Linolenic acid (% dry basis)	***	***	***	8.35^{c}	9.51^{a}	7.83^{d}	8.82^{b}
Oleic acid (% dry basis)	***	***	**	22.7^{d}	28.2^{c}	33.5^{b}	36.4^{a}
Sucrose (% dry basis)	***	***	ns	4.56^{a}	4.49^{a}	4.14^{b}	4.52^{a}
Chlorophyll index of green (CIgreen)	***	***	***	4.03^{a}	4.23^{a}	3.70^{b}	3.67^{b}
Chlorophyll index of red-edge (CIred-edge)	***	***	***	0.93^{a}	0.95^{a}	0.80^{b}	0.77^{b}
Chlorophyll vegetation index (CVI)	***	***	***	2.32^{b}	2.58^{a}	1.92^{c}	1.97^{c}
Normalized Difference Rededge (NDRE)	***	***	***	0.31^{a}	0.32^{a}	0.28^{b}	0.28^{b}
Transformed Chlorophyll Absorption in Reflectance Index (TCARI)	***	***	***	0.21^{a}	0.19^{b}	0.25^{a}	0.24^{a}
Visible Atmospherically Resistant Index (VARI)	***	***	**	1.46^{b}	1.27^{c}	1.77^{a}	1.60^{a}

*, **, and ***, indicate significance levels at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively. 'ns' indicates non-significance. Different letters in superscript indicate the significant treatment effect using the least significant difference (LSD) test at $p < 0.05$.

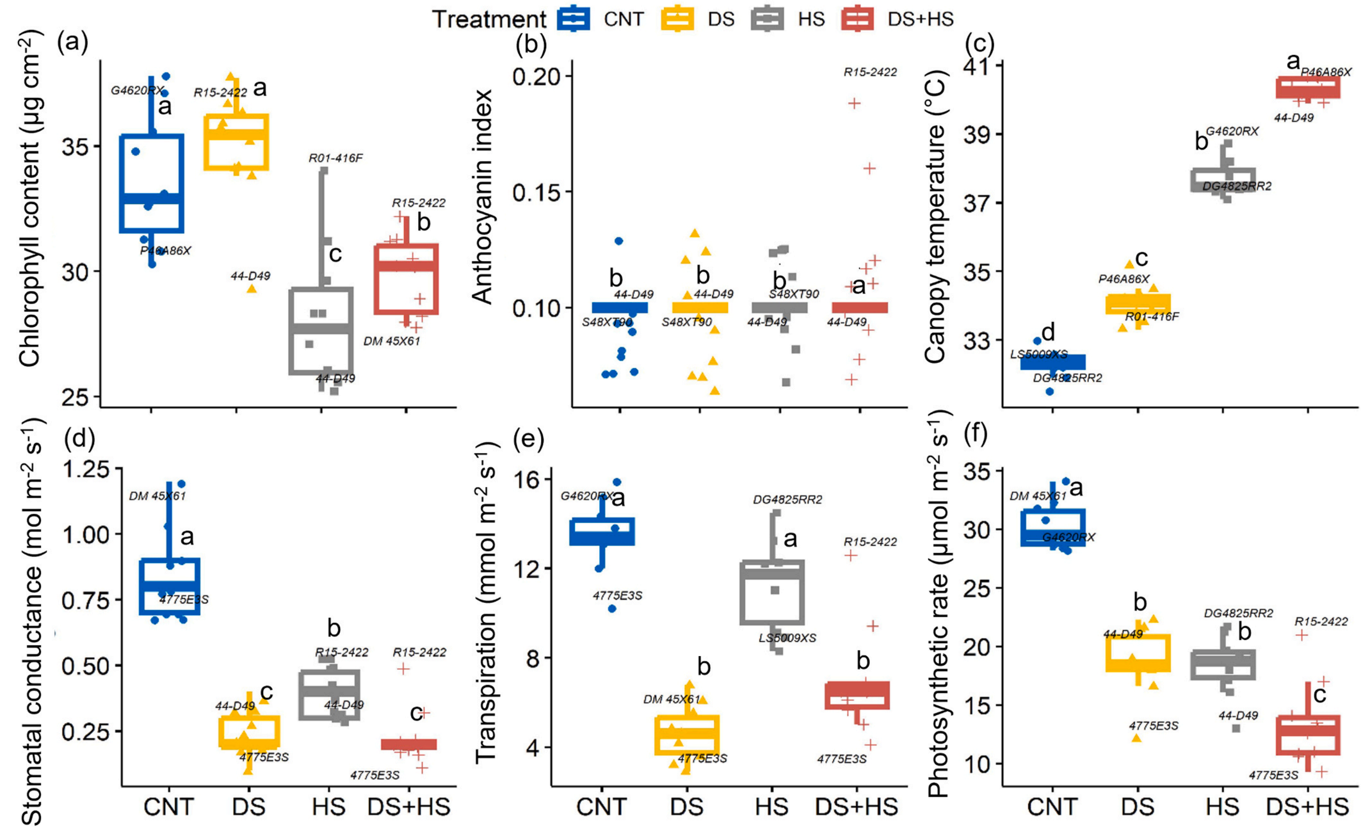

Fig. 2. Effects of drought, heat, and their combination during the flowering to seed-setting on chlorophyll content (a), anthocyanin index (b), canopy temperature (c), stomatal conductance (d), transpiration (e), and photosynthetic rate (f). CNT - control, DS - drought stress, HS - heat stress, and DS+HS - combined drought and heat stress. Means followed by the same letter are not significantly different by the least significant difference (LSD) test at $p < 0.05$.

combined stress. This result suggests that in addition to limited CO_2 availability due to stomatal closure, the canopy temperature increase and leaf pigment changes can further reduce photosynthesis in plants subjected to combined stresses.

3.3. *Leaf spectral properties*

To determine whether individual or combined stress induces different effects on plant health, leaf reflectance properties of all cultivars were measured 14 days after stress. The VIs such as CIgreen, CIred-edge, CVI, and NDRE demonstrated significant variation between the cultivars ($p < 0.001$), treatments ($p < 0.001$), and cultivars × treatments ($p < 0.01$) interaction (Table 1). Under the heat and combined stress, CIgreen, CIred-edge, CVI, and NDRE significantly reduced by 12%, 15%, 15%, and 11% compared to the control, respectively (Table 2). Meanwhile, the VIs CIgreen, CIred-edge, and NDRE did not vary significantly under drought compared to control. In contrast, TCARI and VARI decreased under drought stress (9% and 14%, respectively) and increased under individual heat (21%) and combined stress (18% and 10%, respectively) (Table 2). R01–416F and R15–2422 had the highest VI values under stress conditions, whereas DM45X61, DG4825RR2, and 44-D49 showed the least.

3.4. *Yield parameters*

The yield parameters showed significant variation between treatments ($p < 0.001$, Table 1). Not surprisingly, more pods were observed under control (163 plant^{-1}), followed by heat stress (125 plant^{-1}) compared to combined stress. The drought and combined stress impact on pod number were significantly *at par* with a reduction of ~47% compared to control (Table 1). However, pod weight was reduced by 37% under drought, with the combined stress being the most severe (55% decrease) compared to control. Among the cultivars, the variability in percent reduction in seed yield under individual drought and heat stress ranged from 25–44% and 28–42%, whereas under interactive stress treatment, recorded 53–63% with maximum reduction displayed in 44-D49 and G4620RX compared to control (Fig. 3). Significant seed number and weight reductions were observed under heat (27% and 34%) and drought (49% and 36%). The hundred seed weight was increased significantly by 28% under drought stress compared to control, whereas it was decreased by 8% under heat and 6% under the combined stress (Table 1, Fig. 3). Even though the hundred seed weight under the combined stress was comparable (~36 g) with heat stress, a significant reduction in seed number under the combined stress resulted in a pronounced decrease in seed yield (more than 50%) compared to control (Table 1, Fig. 3). We observed more aborted and empty pods with small, wrinkled seeds under the heat and combined stress, whereas few but bigger seeds were found in the drought-stressed plants (Fig. 3d).

3.5. *Seed quality*

Significant treatment ($p < 0.001$), cultivar ($p < 0.001$), and treatment × cultivar interaction ($p < 0.01$) was observed for protein and oil content (Table 1). All the cultivars responded differentially across the treatments for protein and oil content. Compared to the control, protein content increased by 8% under drought, while oil content decreased by 11%. The protein content was similar to control under individual heat and combined stress. Cultivar R15–2422 had a maximum protein content under drought, heat, and control, and the minimum was observed in DG4825RR2 under individual stress (Fig. 4a). In contrast, the average oil content across the cultivars increased under heat, whereas under combined drought and heat stress, a reduction was observed, with an

Table 2
Variation in leaf reflectance parameters (VIs) of soybean cultivars grown under control (CNT), drought (DS), heat (HS), and combined drought and heat (DS+HS) treatments.

VIs	Cultivar	44-D49	4775E3S	DG4825RR2	DM 45X61	G4620RX	LS5009XS	P46A86X	R01–416F	R15-2422	S48XT90
CIgreen	CNT	3.67±0.17^{a}	3.77±0.17^{ab}	3.65±0.17^{b}	3.68±0.17^{a}	4.27±0.17^{a}	4.11±0.16^{a}	3.94±0.11^{a}	5.38±0.15^{a}	4.07±0.16^{a}	3.75±0.11^{b}
	DS	3.65±0.1^{a}	3.98±0.1^{a}	4.13±0.1^{a}	4±0.1^{a}	4.48±0.1^{a}	4.35±0.15^{a}	4.05±0.12^{a}	4.84±0.16^{ab}	4.29±0.15^{a}	4.47±0.18^{a}
	HS	3.23±0.14^{a}	3.36±0.14^{c}	2.94±0.14^{c}	2.85±0.14^{c}	3.52±0.1^{b}	3.98±0.32^{ab}	3.67±0.19^{ab}	4.45±0.25^{b}	3.85±0.32^{a}	3.55±0.12^{b}
	DS+HS	3.47±0.21^{a}	3.46±0.21^{bc}	3.02±0.21^{c}	3.28±0.21^{bc}	3.58±0.21^{b}	3.49±0.18^{b}	3.58±0.11^{b}	3.61±0.2^{c}	4.05±0.18^{a}	3.6±0.14^{b}
CIred-edge	CNT	0.86±0.04^{a}	0.91±0.04^{a}	0.85±0.04^{a}	0.88±0.04^{a}	1.01±0.04^{a}	0.99±0.04^{a}	0.92±0.02^{a}	1.18±0.02^{a}	0.91±0.04^{a}	0.85±0.02^{b}
	DS	0.81±0.01^{ab}	0.9±0.01^{a}	0.93±0.01^{a}	0.9±0.01^{a}	1.02±0.01^{a}	0.97±0.02^{ab}	0.95±0.03^{a}	1.07±0.04^{b}	0.98±0.02^{a}	1.02±0.04^{a}
	HS	0.73±0.02^{c}	0.77±0.02^{b}	0.69±0.02^{b}	0.66±0.02^{b}	0.8±0.02^{b}	0.9±0.06^{b}	0.81±0.04^{b}	1±0.05^{b}	0.86±0.06^{a}	0.8±0.02^{b}
	DS+HS	0.77±0.04^{bc}	0.77±0.04^{b}	0.69±0.04^{b}	0.73±0.04^{b}	0.8±0.04^{b}	0.76±0.03^{c}	0.8±0.02^{b}	0.77±0.03^{c}	0.88±0.03^{a}	0.79±0.02^{b}
CVI	CNT	2.03±0.14^{a}	2.25±0.08^{a}	1.93±0.22^{b}	2±0.11^{b}	2.7±0.11^{a}	2.43±0.09^{ab}	2.26±0.09^{ab}	3.33±0.19^{a}	2.28±0.19^{b}	1.98±0.08^{b}
	DS	2.01±0.05^{a}	2.36±0.11^{a}	2.35±0.13^{a}	2.32±0.07^{a}	2.86±0.16^{a}	2.71±0.17^{a}	2.53±0.14^{a}	3.22±0.19^{a}	2.74±0.15^{a}	2.76±0.14^{a}
	HS	1.62±0.07^{b}	1.78±0.12^{b}	1.37±0.11^{c}	1.36±0.11^{c}	1.95±0.19^{b}	2.3±0.16^{b}	2.06±0.16^{b}	2.54±0.15^{b}	2.25±0.25^{c}	2.03±0.1^{b}
	DS+HS	1.82±0.14^{ab}	1.9±0.1^{b}	1.67±0.05^{bc}	1.75±0.14^{b}	2.08±0.16^{b}	2.1±0.13^{b}	2.08±0.09^{b}	2.09±0.15^{c}	2.26±0.12^{bc}	2.03±0.1^{b}
NDRE	CNT	0.3±0.01^{a}	0.31±0.01^{a}	0.3±0.01^{a}	0.3±0.01^{a}	0.33±0.01^{a}	0.33±0.01^{a}	0.31±0^{a}	0.37±0^{a}	0.31±0.01^{ab}	0.3±0.01^{b}
	DS	0.29±0^{ab}	0.31±0^{a}	0.32±0^{a}	0.31±0^{a}	0.34±0^{a}	0.33±0.01^{ab}	0.32±0.01^{a}	0.35±0.01^{ab}	0.33±0.01^{a}	0.34±0.01^{a}
	HS	0.27±0.01^{c}	0.28±0.01^{b}	0.25±0.01^{b}	0.25±0.01^{b}	0.28±0.01^{b}	0.31±0.02^{b}	0.29±0.01^{b}	0.33±0.01^{b}	0.3±0.02^{b}	0.28±0.01^{b}
	DS+HS	0.28±0.01^{bc}	0.28±0.01^{b}	0.26±0.01^{b}	0.26±0.01^{b}	0.28±0.01^{b}	0.27±0.01^{c}	0.28±0^{b}	0.28±0.01^{c}	0.31±0.01^{ab}	0.28±0.01^{b}
TCARI	CNT	0.22±0.02^{b}	0.2±0.02^{b}	0.25±0.02^{b}	0.21±0.02^{bc}	0.17±0.02^{b}	0.18±0.01^{b}	0.2±0.01^{ab}	0.13±0.01^{c}	0.2±0.01^{a}	0.22±0.01^{a}
	DS	0.22±0.01^{b}	0.2±0.01^{b}	0.19±0.01^{c}	0.19±0.01^{c}	0.17±0.01^{b}	0.17±0.01^{b}	0.18±0.01^{b}	0.15±0.01^{c}	0.17±0.01^{a}	0.16±0.01^{b}
	HS	0.26±0.01^{a}	0.25±0.01^{a}	0.31±0.01^{a}	0.31±0.01^{a}	0.24±0.01^{a}	0.19±0.03^{b}	0.22±0.02^{a}	0.18±0.01^{b}	0.22±0.03^{a}	0.21±0.01^{a}
	DS+HS	0.24±0.02^{ab}	0.23±0.02^{a}	0.28±0.02^{ab}	0.26±0.02^{ab}	0.22±0.02^{a}	0.23±0.01^{b}	0.22±0.01^{a}	0.22±0.01^{a}	0.21±0.01^{a}	0.22±0.01^{a}
VARI	CNT	1.42±0.08^{b}	1.24±0.08^{b}	1.72±0.08^{b}	1.47±0.08^{b}	1.11±0.08^{b}	1.19±0.15^{ab}	1.38±0.04^{a}	1.09±0.11^{bc}	1.49±0.15^{a}	1.57±0.09^{a}
	DS	1.42±0.05^{b}	1.26±0.05^{b}	1.35±0.05^{b}	1.29±0.05^{b}	1±0.05^{b}	1.14±0.05^{b}	1.12±0.06^{b}	0.95±0.07^{c}	1.1±0.05^{b}	1.14±0.08^{b}
	HS	1.75±0.09^{a}	1.72±0.09^{a}	2.25±0.09^{a}	2.14±0.09^{a}	1.62±0.09^{a}	1.41±0.15^{a}	1.57±0.11^{a}	1.32±0.1^{ab}	1.49±0.15^{a}	1.48±0.08^{a}
	DS+HS	1.68±0.13^{ab}	1.57±0.13^{a}	1.61±0.13^{b}	1.69±0.13^{b}	1.42±0.13^{a}	1.33±0.07^{ab}	1.43±0.09^{a}	1.38±0.08^{a}	1.43±0.07^{a}	1.48±0.09^{a}

Values represent mean (n = 10) ± SE for the leaf reflectance parameters (VIs). Different letters in superscript indicate the significant treatment effect for a given parameter between treatments. The mean values were separated using the least significant difference (LSD) test at $p < 0.05$.

exception noticed in the cultivars; R15–2422, R01–416F, and DG4825RR2, and the least was in 44-D49 (Fig. 4b).

Soybean is a rich source of linoleic and linolenic acids. However, when exposed to stressors, the composition of these essential fatty acids changed significantly. For example, linoleic acid, the most abundant unsaturated fatty acid in soybean, decreased under all stressors. The maximum reduction was recorded under combined stress (25%), while the minimum reduction occurred under drought (12%) (Fig. 4c).

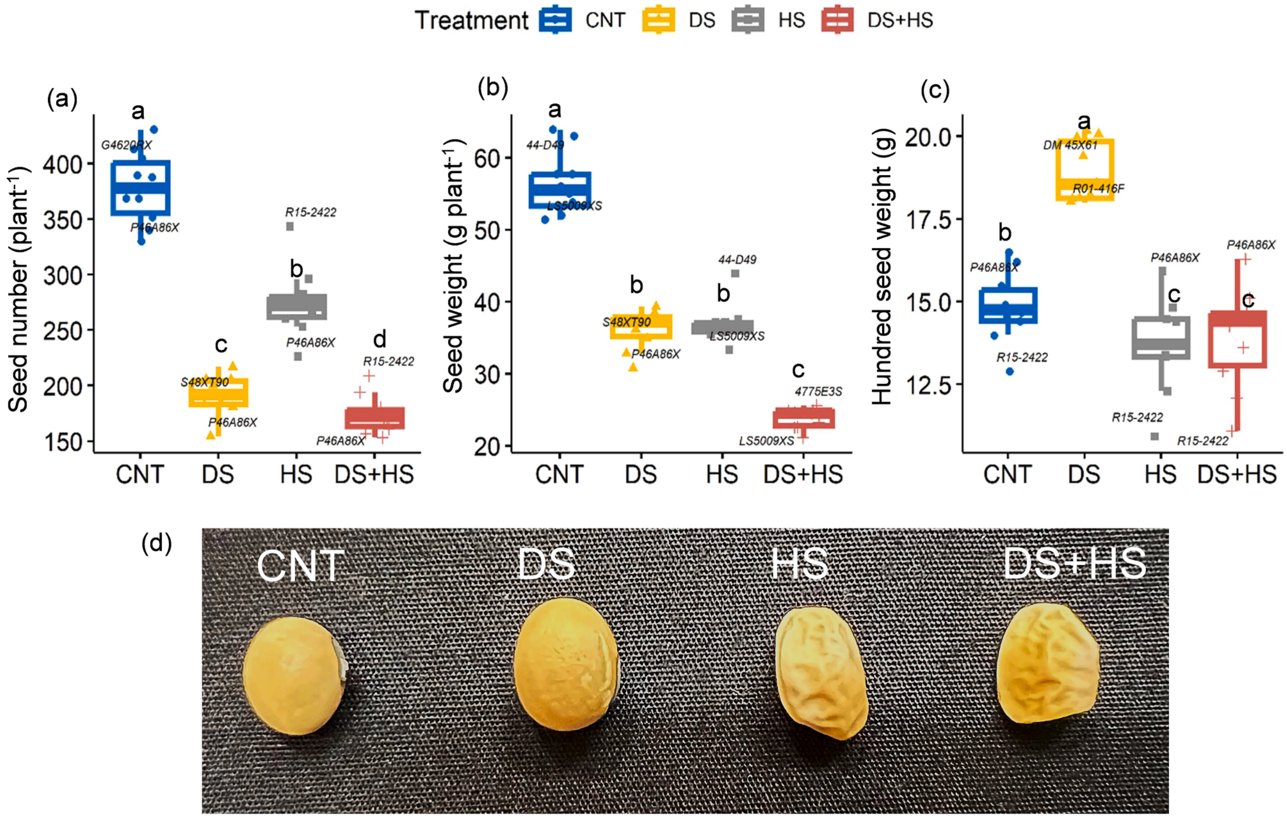

Fig. 3. Effects of drought, heat, and combined drought and heat stresses on seed number (a), seed weight (b), and hundred seed weight (c). Pictorial representation of individual seed size under four treatment conditions (d). CNT - control, DS - drought stress, HS - heat stress, and DS+HS - combined drought and heat stress. Means followed by the same letter are not significantly different by the least significant difference (LSD) test at $p < 0.05$.

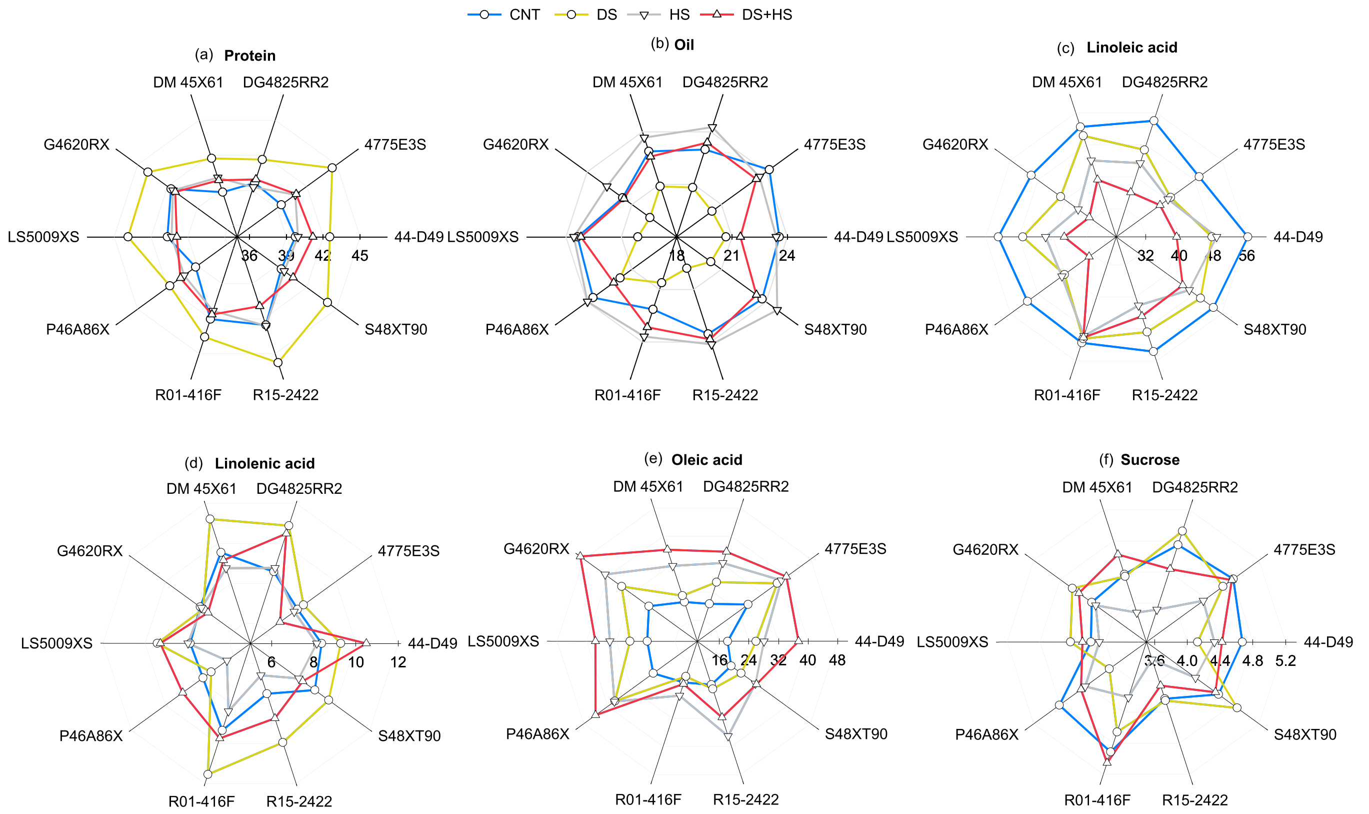

Fig. 4. Effects of drought, heat, and combined stresses on protein (% dry basis, a), oil (% dry basis, b), linoleic acid (% dry basis, c), linolenic acid (% dry basis, d), oleic acid (% dry basis, e), and sucrose (% dry basis, f). CNT - control, DS - drought stress, HS - heat stress, and DS+HS - combined drought and heat stress.

Although soybean contain lower amounts of linolenic acid than linoleic acid, there was a significant increase in linolenic acid under drought and combined stress compared to the control. On the other hand, the monounsaturated fatty acid; oleic acid, showed a higher accumulation under combined stress, followed by individual drought and heat stress (Table 1; Fig. 4). Under individual and combined stress, the cultivars with the highest linoleic content, R01–416F, and R15–2422, exhibited a reduced level of oleic acid (Fig. 4e). In contrast, the opposite is true, indicating a discernible trade-off between these fatty acids. The stress tolerance and yield of soybean depend on this tradeoff and the interaction between protein and oil content.

3.6. *Gene expression response of stress-responsive genes in soybean to stressors*

GLYMA.07G109100 encodes a pentatricopeptide repeat-containing protein (PRR) that has a protective role in scavenging reactive oxygen species (ROS) specific to heat stress (Xu et al., 2019). However, less variation among the heat-stressed group was observed among ten cultivars, whereas drought-stressed 4775E3S, P46A86X, R15–2422, and combined stressed DM45X61 displayed a significantly higher transcript level compared to control (Fig. 5). The lack of significant changes across treatments may suggest the malfunction of oxidoreductase following long-term stress, resulting in a reduced ability to remove ROS. Additionally, GLYMA.03G30040, a homolog of AT5G06760, which encodes late embryogenesis abundant proteins, exhibited significant induction under individual drought and combined stress. Among the cultivars, gene expression was significantly induced under drought compared to control for 44-D49, DM45X61, G4620RX, LS5009XS, and R15–2422. Additionally, S48XT90 exhibited a substantial induction of gene expression under combined stress (Fig. 5).

3.7. *Stress tolerance index*

The stress tolerance index (STI) was used to determine soybean cultivars' tolerance to individual and combined stresses. The cultivar DM45X61 displayed higher tolerance, followed by 44-D49 and R15–2422 under drought and heat stress for the physiological parameters (Fig. 6). Under combined stress, the cultivar 44-D49 had a higher tolerance rank for the physiological parameters, followed by G4620RX and R15–2422 (Fig. 6). The cultivar R01–416F had higher tolerance in terms of leaf reflectance properties, consistently demonstrating higher quality and gene expression rankings across all three treatments, except for yield traits.

For the yield parameters, cultivar 44-D49 was tolerant under drought and heat stress (Fig. 6). However, the cultivar DM45X61, which performed better in terms of physiology under individual drought and heat, did not show the highest rank for yield, possibly due to the negative impacts of the stress on the pollen viability and reproductive failure (Bheemanahalli et al., 2019; Poudel et al., 2023b). Meanwhile, under combined stress, the cultivar G4620RX, with the highest tolerance for physiological performance, showed better ranking for yield parameters (Fig. 6). Based on the average tolerance rank across the parameters, the cultivars R15–2422, G4620RX, and 44-D49 were the tolerant cultivars across the treatments. Similarly, the cultivar R01–416F, followed by 4775E3S, had the highest stress tolerance rank for quality traits and gene expression under the individual and combined drought and heat stress (Fig. 6).

4. Discussion

A paradigm shift occurring under combined drought and heat stress compared to individual stressors is gaining prominence in beans and other crops (Zandalinas et al., 2018; Ergo et al., 2018; Lawas et al., 2018; Zandalinas et al., 2020; Cohen et al., 2021a; b; Bheemanahalli et al.,

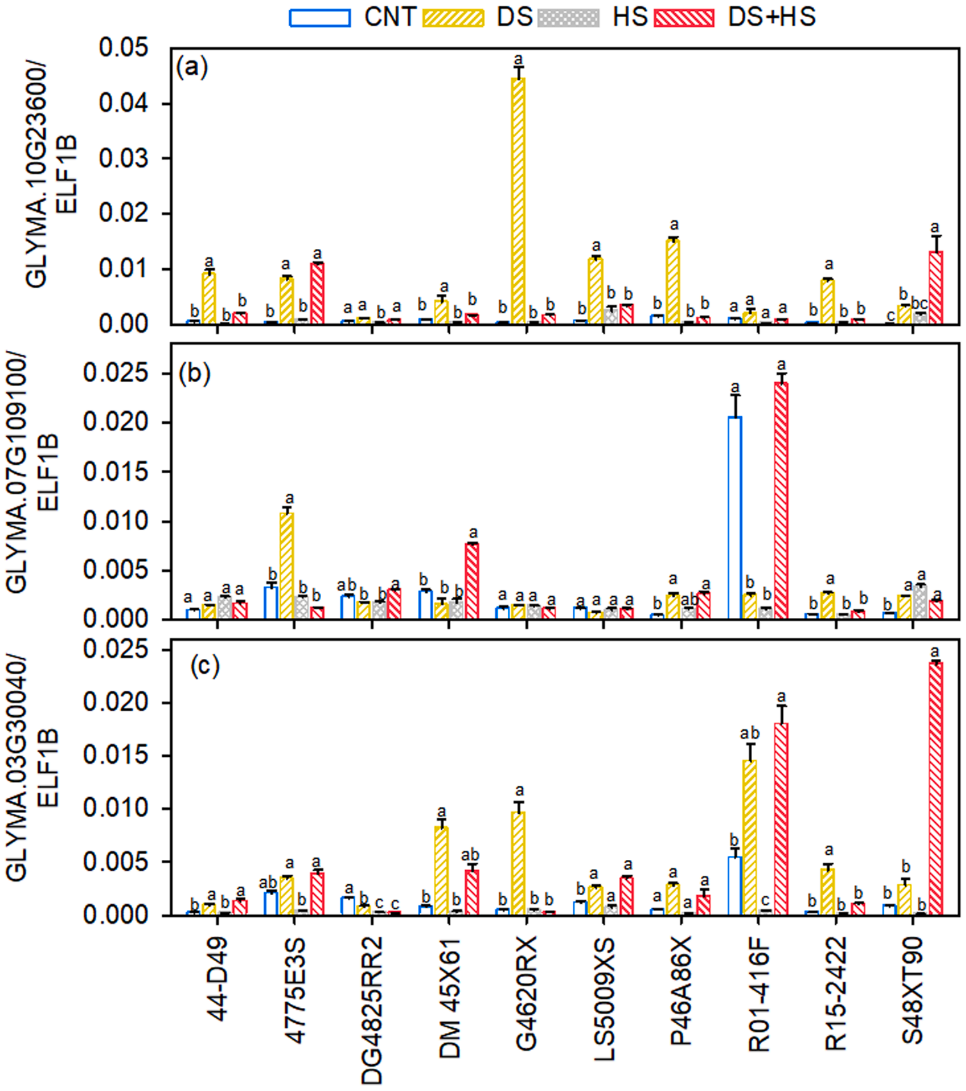

Fig. 5. Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) of stress marker genes during the seed-setting stage under various stress conditions. The test was conducted using stress-responsive genes, which include (a) the drought-responsive gene GLYMA.10G23600, (b) the heat-responsive gene GLYMA.07G109100, and (c) the combined stress-responsive gene GLYMA.03G30040. The data represent mean values and standard errors derived from four biological replicates. A cultivar with different letters indicates significant differences within the cultivar under different stress based on the Tukey HSD test at $p < 0.05$.

2022a). Recent research shows a synergistic effect of combined drought and heat stress, particularly affecting source-sink balance and yield parameters (Du et al., 2023). The present results show that interactive drought and heat had a higher negative impact on physiology, yield, and seed composition than single stress at the cultivar level in soybeans.

4.1. Interactive stress-induced changes in physiology and leaf reflectance properties in soybean

Combined stress resulted in a synergistic adverse effect on plant traits with a greater negative impact on the physiological traits than individual stress (Fig. 2). Drought stress significantly lowers stomatal conductance, reducing transpiration rate (Figs. 2, 8). Notably, the drought-stressed plants maintained greenness similar to the control (Fig. 2a; Supplementary Fig. S1a), indicating that plants may prioritize chlorophyll production even under drought stress to maintain their ability to capture sunlight and convert into chemical energy (Jurik, 1986). Likewise, heat-stressed plants increased their transpiration rate (Fig. 2e) to maintain a cooler canopy (Sinha et al., 2022; Poudel et al., 2023a). To cope with interactive drought and heat, plants selectively close their leaf stomata to conserve water while keeping flower and pod stomata open (Cohen et al., 2021b; Sinha et al., 2022). Compared to vegetative tissue, reproductive tissue showed higher transpiration as a avoidance mechanism against overheating (Sinha et al., 2022; Vennam et al., 2023b). Combined stress triggered a significant rise in canopy temperature by 8 °C, a key indicator of plant water status. This rise corresponded with a marked disruption in photosynthesis and stomatal conductance, aligning with previous studies (Cohen et al., 2021b; Bheemanahalli et al., 2022a).

Significant differences in leaf reflectance properties between treatments indicate the changes in pigment accumulation; this response is often used to determine plant health. The study found that tolerant cultivars had a higher reflectance near-infrared region (NIR, lower absorbance) than sensitive cultivars (Supplementary Fig. 2), possibly due to changes in leaf structure or leaf water content (Bowman, 1989; Zhang et al., 2012). Further, the VIs associated with greenness, such as CIgreen, CIred-edge, CVI, and NDRE, were comparable with manual measurements under stress (Lima et al., 2020; Aldubai et al., 2022). Differential response of VIs between control and treatments among cultivars indicates greater variability in stress tolerance (Table 2). Among the VIs, we observed a significant decrease in CIred-edge across the ten soybean cultivars under combined stress (Table 2). The

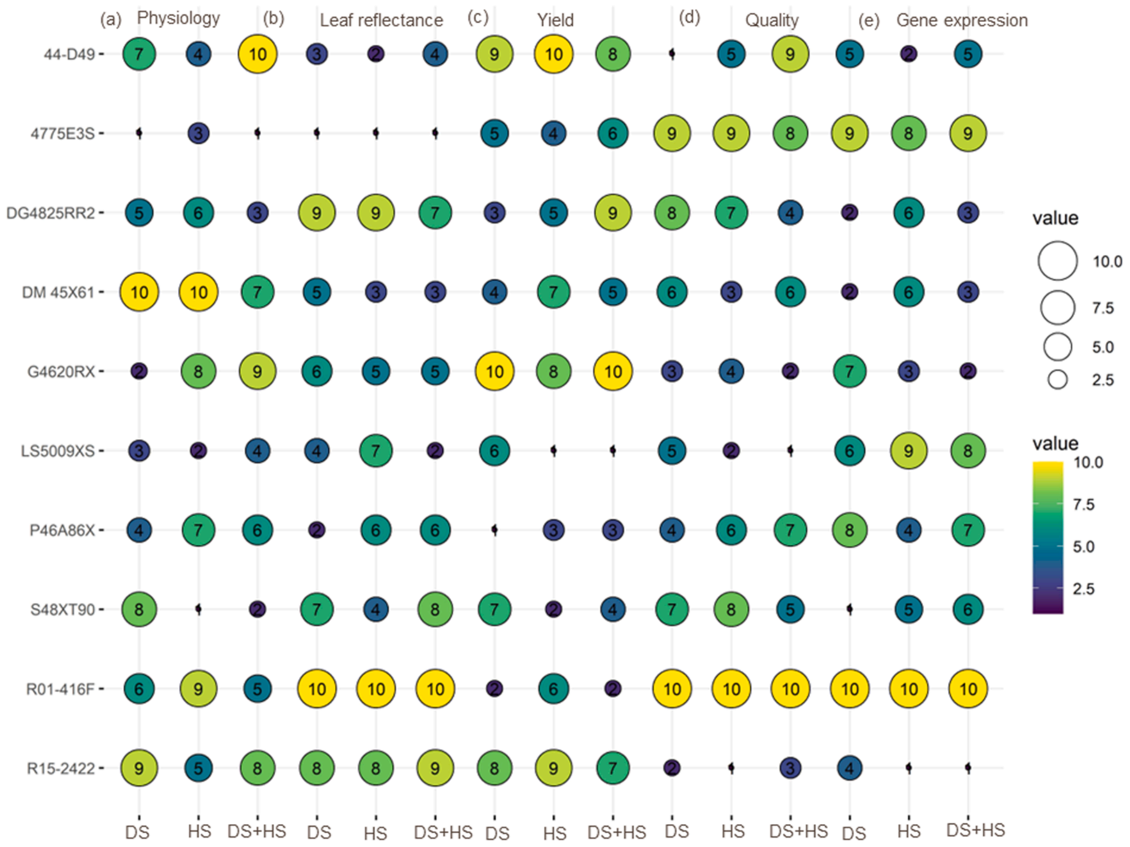

Fig. 6. Bubble plot showing the average stress tolerance index values for physiology (chl, anth, gs, E, CT, A; a), leaf reflectance (CI green, CI red-edge, CVI, NDRE, TCARI, VARI; b), yield (PN, PWt., SN, SWt., HSWt.; c), seed quality (protein, oil, linoleic acid, linolenic acid, oleic acid, sucrose; d), and gene expression (drought-responsive gene GLYMA.10G23600, heat-responsive gene GLYMA.07G109100, combined stress-responsive gene GLYMA.03G30040; e). A cultivar with a larger bubble size (yellow color) indicates higher stress tolerance and *vice versa*. DS - drought stress, HS - heat stress, and DS+HS - combined drought and heat stresses. Acronyms are given in Table 1.

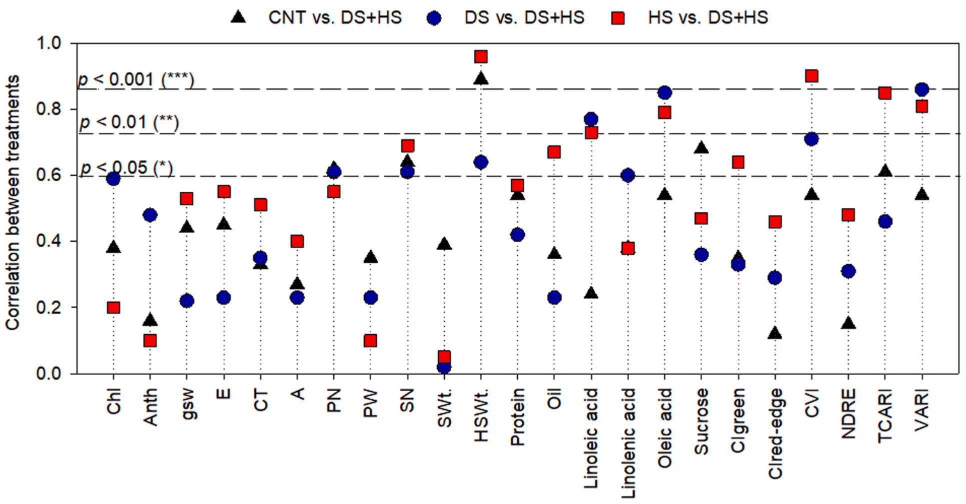

Fig. 7. Correlation of traits between single and combined stress treatments. *, **, and ***, indicate significance levels at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively. CNT - control, DS - drought stress, HS - heat stress, and DS+HS - combined drought and heat stresses. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) indicate a significant correlation between treatment for a given trait. Traits acronyms are given in Table 1.

CIred-edge reflects chlorophyll content and physiological status in plants. This also supports the hypothesis that stress-induced changes in the absorption, reflectance, and transmittance of radiation from the leaf vary with genetics (Walter-Shea et al., 1991; Kataria et al., 2014). As reported in other crops (Bheemanahalli et al., 2022a; Brewer et al., 2022), changes observed with the VIs suggest that proximal sensing can used to quantify the impact of stressors in soybean. Unlike single stress, combined stress did not show a significant difference in NIR between tolerant and sensitive cultivars (Supplementary Fig. 2). Although NIR reflectance has been established as a dependable tool for evaluating plant responses and distinguishing between tolerant and sensitive species to individual drought and heat stress, additional research is necessary to understand the interplay between various stressors and crops.

4.2. The implication of stress-responsive genes into phenotypic performance

The interplay of genes regulating hormonal signaling and regulatory pathways governs the intricate physiological and morphological adjustments in response to various abiotic stresses (Zhang et al., 2022; Kumar et al., 2023). Among these, GLYMA.10G23600, analogous to Arabidopsis RD29A and RD29B genes, is involved in the abscisic acid (ABA) signaling pathway, which is crucial for orchestrating plant responses to drought stress (Msanne et al., 2011). Abscisic acid plays a key role in modulating stomatal movement, transpiration, and chlorophyll degradation, underscoring its significance in supporting stress tolerance and shaping physiological processes (Hsu et al., 2021; Bharath et al., 2021). The observed correlation between GLYMA.10G23600 expression levels and stress-related phenotypes in cultivars like G4620RX and S48XT90 suggests a potential for enhanced stress response through modulated ABA signaling. Additionally, GLYMA.07G109100 encodes a pentatricopeptide repeat-containing protein essential for scavenging reactive oxygen species (ROS) under heat stress conditions (Yu et al., 2021b). Prolonged heat stress can lead to excess ROS that exceeds cultivar's capacity to maintain redox signaling (Fortunato et al., 2023). Under drought, some cultivars show high gene expression, indicating effective ROS scavenging during prolonged drought or combined stress. Conversely, while heat stress exhibited less variation among cultivars, drought-stressed cultivars such as 4775E3S, P46A86X, R15–2422, and combined-stress-exposed DM45X61, displayed a significantly higher transcript level compared to control (Fig. 5). This observation may imply potential malfunction of oxidoreductase pathways following prolonged stress, leading to compromised ROS removal capabilities. Furthermore, GLYMA.03G30040, is identified as a drought and heat stress marker homologous to Arabidopsis late embryogenesis abundant (LEA) proteins. Most LEA proteins are considered a subset of hydrophilies with a specialized function in retaining water molecules (Battaglia et al., 2008). Previous studies showed its induction during short-term stress in soybean (Wang et al., 2018; Guo et al., 2023). However, its expression patterns during long-term stress are not well-characterized. The current study demonstrated elevated expression of GLYMA.03G30040 in drought-stressed cultivars, suggesting LEA accumulation as a response to prolonged stress. Various cultivars show significant gene expression alterations under drought stress, including 44-D49, DM45X61, G4620RX, LS5009XS, and R15–2422. On the other hand, S48XT90 had pronounced induction under combined stress conditions. This highlights the complex genetic basis of stress adaptation mechanisms and the diverse transcript alterations in stress-responsive genes across cultivars.

4.3. Interactive stress-induced alterations in yield components

The negative impact of combined stress on yield was two-fold higher than drought or heat alone (Fig. 3), with a similar response recorded in other crops (Bheemanahalli et al., 2022a). This suggests that the final yield is a complex integration of physiological, biochemical, and phenological processes (Fig. 8). Despite a significant difference in seed number between drought and heat, seed weight remains similar. This suggests drought stress severely impacts reproductive potential (nodes and pod number) more than heat stress (Poudel et al., 2023a). Although drought stress decreased the number of pods (37%) and seeds (49%), the hundred seed weight was higher than other stressors. This could be due to the efficient translocation of photosynthates to a limited number of active pods and seeds (Ney et al., 1994; Poudel et al., 2023b). Despite having the least reduction in seed number under single stress (Fig. 3 a), R15–2422 exhibited a significant decline in hundred seed weight under combined stress (Fig. 3c). The seed number and weight tradeoff has been a well-documented adaptive strategy in crops (Griffiths et al., 2015; Cohen et al., 2021b). Heat stress alone or combined with drought-induced early senescence and shortened seed-filling duration resulted in fewer pods with small, wrinkled seeds (Fig. 3d; Pradhan et al., 2012; Jumrani and Bhatia, 2018). This suggests an imbalance in resource translocation from source to sink organs. In addition, the higher number of empty pods and aborted seeds observed under heat and combined stress suggests the sensitivity of reproductive failure, as reported in other studies (Koti et al., 2005; Bheemanahalli et al., 2022a; Sinha et al., 2022). Currently, soybean cultivars are bred for a higher yield. However, none of these cultivars can tolerate combined stress during reproductive and seed-filling. Therefore, a particular focus should be given to developing cultivars with superior physiological traits that minimize reproductive failure to achieve higher yields under combined stress.

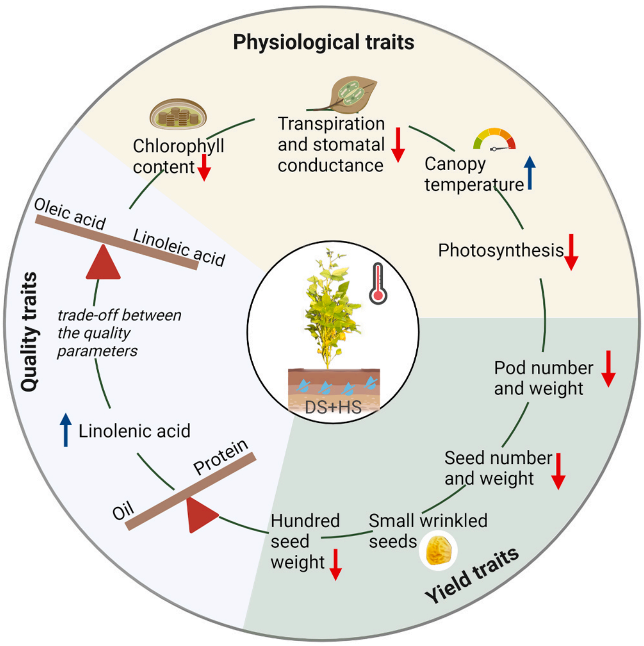

Fig. 8. Summary of combined drought and heat stress (DS+HS) impact on soybean morpho-physiology, yield, and quality traits in soybean. Illustration created using the Biorender.

4.4. Trade-off between yield and seed quality components

Protein and oil content showed an inverse correlation across treatments, with a stronger negative correlation under drought, consistent with other studies (Bellaloui et al., 2015; Mourtzinis et al., 2017; Bheemanahalli et al., 2022b). The decrease in seed weight under combined stress is attributed to the reduced availability of substrate, which results in a tradeoff between protein and oil content. Heat stress during grain development was observed to reduce lipid unsaturation levels, which negatively impacts the nutritional quality of soybeans by reducing essential fatty acids such as linoleic acid and linolenic acid (Bukowski and Goslee, 2024). Studies have shown that increased oleic acid accompanies a decline in polyunsaturated fatty acid and linoleic acid under combined stress (Bellaloui et al., 2015). The R01–416F and R15–2422 cultivars had a high amount of linoleic acid, while the P46A86X and G4620RX had the lowest amount. The study also revealed that the cultivar with the least linoleic acid content had low yield and higher oleic acid (Fig. 4). It was found that the alteration in linoleic and oleic acid was attributed to the activation of the Triacylglycerol (TAG) degradation pathway, specifically, the reduced activity of desaturase enzymes under combined stress (Fehr, 2007; Bellaloui et al., 2015; Assefa et al., 2018; Kanai et al., 2019). While increased oleic acid levels and decreased linoleic and linolenic acid levels enhance the oxidative stability of the oil and result in more acceptable flavor quality scores, it is important to note that these polyunsaturated fatty acids are known for their ability to lower cholesterol levels in human blood, thereby reducing the risk of heart diseases (Agyenim-Boateng et al., 2023). The cultivar with the highest yield (4775E3S) had higher seed protein under combined drought and heat than control but reduced oil content. This observation aligns with previous studies, which show that drought and heat combined result in high seed protein content, providing an advantage to legumes over cereals in tolerating these stressors (Cohen et al., 2021a). However, cultivars with superior physiological traits showed a higher percentage reduction in protein than the control. Enhancing the quality of soybeans without reducing yield can be achieved by manipulating lipase activity (Kanai et al., 2019). This finding indicates that interactive stress further increases the complexity of a tradeoff in yield and quality traits (Fig. 8).

4.5. Individual and combined stress treatments revealed unique traits relationships

When plants experience simultaneous drought and heat stresses, their adaptation strategy is not just the sum of individual responses. Rather, it is influenced by the interaction of these stresses, perceived by plants as a new and unique state of stress (Mittler, 2006; Pandey et al., 2015). This results in different adaptation strategies under combined stress compared to individual stress. In our study, correlation analyses explained the unique and shared traits relationship between individual and combined treatments (Fig. 7). We observed that the combined effect of drought and heat stress has a more severe impact on physiological processes than each stress alone. The physiological parameters (stomatal conductance, transpiration, canopy temperature, photosynthesis) did not show significant correlations between control or single and combined stress (Fig. 7). In contrast to hundred seed weight and pod number, all yield-related parameters showed weaker correlations between individual and combined stress treatments. This indicates greater plasticity in traits response to individual and combined stress. Under combined stress, some trait responses were similar to drought or heat stress. For instance, drought and combination reduced the pod number, unaffected by heat stress. Similarly, heat and combined stress increased the number of aborted, wrinkled, and small seeds, whereas drought stress did not reduce seed size. This suggests that selecting cultivars for stress tolerance based on individual stress performance may not accurately reflect resilience under combined stress. Our study, in line with similar observations in tobacco (Rizhsky et al., 2002) and Arabidopsis (Rizhsky et al., 2004), found that plant responses to combined stress are not directly comparable to responses evoked by individually imposed stress and *vice-versa*. This is likely because the combination of drought and heat stress triggers different, sometimes conflicting (e.g., *gs*) signaling pathways than the individual stresses (Rizhsky et al., 2004). This can lead to synergistic negative effects, potentially reflecting a highly elevated stress level in the combined scenario beyond the impact of single stresses. Therefore, understanding the individual and compound effects of stressors is crucial for selecting tolerant cultivar in soybeans and other crops.

5. Conclusion

The individual drought or heat and combined stress-induced genetic variability in physiological, yield, and quality traits were explored. Overall, our study highlights that the soybean cultivars were more susceptible to combined drought and heat than the individual stresses. Analyses of ten cultivars' performance revealed a complex interplay between the individual and combined stressors. Contrary to prior assumptions, resilience to individual stressors did not consistently perform under combined stress for all measured traits. This suggests that cultivar selection for multi-stress environments requires a multifaceted approach, considering specific stress combinations and their intricate impact on plant physiology, yield, and quality. This research highlights the need for understanding cultivar-specific responses to combined stresses at the reproductive stage for developing stress-tolerant cultivars.

Funding information

This research was funded by the Mississippi Soybean Promotion Board (MSPB, #43 to RB), the USDA-Agricultural Research Service (USDA-ARS: 58-6066-2-031 to RB), NSF award IOS-2038872 to MSM and the National Institute of Food and Agriculture (MIS 043050).

CRediT authorship contribution statement

Sadikshya Poudel: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Ranadheer Reddy Vennam** : Writing – review & editing, Data curation. **Lekshmy V. Sankarapillai**: Writing – review & editing, Formal analysis. **Jinbao Liu**: Writing – review & editing, Data curation. **K. Raja Reddy**: Writing – review & editing. **Nuwan K. Wijewardane**: Writing – review & editing, Formal analysis. **M. Shahid Mukhtar**: Writing – review & editing. **Raju Bheemanahalli**: Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition.

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.

Acknowledgments

We appreciate the Plant Stress Physiology Lab team for assisting with data collection. We thank David Brand for his technical support during the experiment. The mention of trade names or commercial products in this publication solely provides specific information and does not imply recommendation or endorsement.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2024.105769.

References

Agyenim-Boateng, K.G., Zhang, S., Zhang, S., Khattak, A.N., Shaibu, A., et al., 2023. The nutritional composition of the vegetable soybean (Maodou) and its potential in combatting malnutrition. Front. Nutr. 9 https://www.frontiersin.org/articles/10.3389/fnut.2022.1034115 (accessed 8 January 2024).

Aldubai, A.A., Alsadon, A.A., Al-Gaadi, K.A., Tola, E., Ibrahim, A.A., 2022. Utilizing spectral vegetation indices for yield assessment of tomato genotypes grown in arid conditions. Saudi J. Biol. Sci. 29 (4), 2506–2513. https://doi.org/10.1016/j.sjbs.2021.12.030.

Alsajri, F.A., Wijewardana, C., Bheemanahalli, R., Irby, J.T., Krutz, J., et al., 2022. Morpho-physiological, yield, and transgenerational seed germination responses of soybean to temperature. Front. Plant Sci. 13 https://www.frontiersin.org/articles/10.3389/fpls.2022.839270 (accessed 10 December 2022).

Annual Soy Stats Results. 2021. Am. Soybean Assoc. https://soygrowers.com/education-resources/publications/soy-stats/ (accessed 29 November 2021).

Assefa, Y., Bajjalieh, N., Archontoulis, S., Casteel, S., Davidson, D., et al., 2018. Spatial characterization of soybean yield and quality (amino acids, oil, and protein) for United States. Sci. Rep. 8 (1), 14653 https://doi.org/10.1038/s41598-018-32895-0.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Battaglia, M., Olvera-Carrillo, Y., Garciarrubio, A., Campos, F., Covarrubias, A.A., 2008. The enigmatic lea proteins and other hydrophilins. Plant Physiol. 148 (1), 6–24. https://doi.org/10.1104/pp.108.120725.

Bellaloui, N., Bruns, H.A., Abbas, H.K., Mengistu, A., Fisher, D.K., et al., 2015. Agricultural practices altered soybean seed protein, oil, fatty acids, sugars, and minerals in the Midsouth USA. Front. Plant Sci. 6 https://doi.org/10.3389/fpls.2015.00031.

Bharath, P., Gahir, S., Raghavendra, A.S., 2021. Abscisic acid-induced stomatal closure: an important component of plant defense against abiotic and biotic stress. Front. Plant Sci. 12, 615114 https://doi.org/10.3389/fpls.2021.615114.

Bheemanahalli, R., Poudel, S., Alsajri, F., Reddy, K., 2022b. Phenotyping of southern United States soybean cultivars for potential seed weight and seed quality compositions. Agronomy 12. https://doi.org/10.3390/agronomy12040839.

Bheemanahalli, R., Ramamoorthy, P., Poudel, S., Samiappan, S., Wijewardane, N., et al., 2022a. Effects of drought and heat stresses during reproductive stage on pollen germination, yield, and leaf reflectance properties in maize (*Zea mays* L. Plant Direct 6 (8), e434. https://doi.org/10.1002/pld3.434.

Bheemanahalli, R., Sunoj, V.S.J., Saripalli, G., Prasad, P.V.V., Balyan, H.S., et al., 2019. Quantifying the impact of heat stress on pollen germination, seed set, and grain filling in spring wheat. Crop Sci. 59 (2), 684–696. https://doi.org/10.2135/cropsci2018.05.0292.

Bowman, W.D., 1989. The relationship between leaf water status, gas exchange, and spectral reflectance in cotton leaves. Remote Sens. Environ. 30 (3), 249–255. https://doi.org/10.1016/0034-4257(89)90066-7.

Brewer, K., Clulow, A., Sibanda, M., Gokool, S., Naiken, V., et al., 2022. Predicting the chlorophyll content of maize over phenotyping as a proxy for crop health in smallholder farming systems. Remote Sens 14 (3), 518. https://doi.org/10.3390/rs14030518.

Bukowski, M.R., Goslee, S., 2024. Climate-based variability in the essential fatty acid composition of soybean oil. Am. J. Clin. Nutr. 119 (1), 58–68. https://doi.org/10.1016/j.ajcnut.2023.08.024.

Chen, P., Sneller, C.H., Purcell, L.C., Sinclair, T.R., King, C. a, et al., 2007. Registration of soybean germplasm lines R01–416F and R01–581F for improved yield and nitrogen fixation under drought stress. J. Plant Regist. 1 (2), 166–167. https://doi.org/10.3198/jpr2007.01.0046crg.

Cohen, I., Huck, C., Fritschi, F.B., Mittler, R., Zandalinas, S.I., 2021a. Meta-analysis of drought and heat stress combination impact on crop yield and yield components. Physiol. Plant. 171 (1), 66–76. https://doi.org/10.1111/ppl.13203.

Cohen, I., Zandalinas, S.I., Fritschi, F.B., Sengupta, S., Fichman, Y., et al., 2021b. The impact of water deficit and heat stress combination on the molecular response, physiology, and seed production of soybean. Physiol. Plant. 172 (1), 41–52. https://doi.org/10.1111/ppl.13269.

Du, X., Zhang, X., Wei, Z., Lei, W., Hu, G., et al., 2023. Photosynthetic characteristics of subtending leaves and their relationships with soybean pod development under heat, drought and combined stresses. J. Agron. Crop Sci. 209 (1), 204–215. https://doi.org/10.1111/jac.12616.

Ergo, V.V., Lascano, R., Vega, C.R.C., Parola, R., Carrera, C.S., 2018. Heat and water stressed field-grown soybean: a multivariate study on the relationship between physiological-biochemical traits and yield. Environ. Exp. Bot. 148, 1–11. https://doi.org/10.1016/j.envexpbot.2017.12.023.

Fehr, W.R., 2007. Breeding for modified fatty acid composition in soybean. S-72-S-87 Crop Sci. 47 (S3). https://doi.org/10.2135/cropsci2007.04.0004IPBS.

Fehr, W.R., and C.E. Caviness. 1977. Stages of soybean development. Special Report 80, Iowa Agricultural Experiment Station, Iowa Cooperative External Series, Iowa State University, Ames.

Fernandez, G.C.J., 1992. Stress tolerance index- a new indicator of tolerance. HortScience 27 (6), 626d–6626d. https://doi.org/10.21273/HORTSCI.27.6.626d.

Fortunato, S., Lasorella, C., Dipierro, N., Vita, F., de Pinto, M.C., 2023. Redox signaling in plant heat stress response. Antioxidants 12 (3), 605. https://doi.org/10.3390/antiox12030605.

Gitelson, A.A., Kaufman, Y.J., Stark, R., Rundquist, D., 2002. Novel algorithms for remote estimation of vegetation fraction. Remote Sens. Environ. 80 (1), 76–87. https://doi.org/10.1016/S0034-4257(01)00289-9.

Gitelson, A.A., Viña, A., Arkebauer, T.J., Rundquist, D.C., Keydan, G., et al., 2003. Remote estimation of leaf area index and green leaf biomass in maize canopies. Geophys. Res. Lett. 30 (5) https://doi.org/10.1029/2002GL016450.

Griffiths, S., Wingen, L., Pietragalla, J., Garcia, G., Hasan, A., et al., 2015. Genetic dissection of grain size and grain number trade-offs in cimmyt wheat germplasm. PLoS One 10 (3), e0118847. https://doi.org/10.1371/journal.pone.0118847.

Guo, B., Zhang, J., Yang, C., Dong, L., Ye, H., et al., 2023. The late embryogenesis abundant proteins in soybean: identification, expression analysis, and the roles of GmLEA4_19 in drought stress. Int. J. Mol. Sci. 24 (19), 14834. https://doi.org/10.3390/ijms241914834.

Haboudane, D., Miller, J.R., Tremblay, N., Zarco Tejada, P.J., Dextraze, L., 2002. Integrated narrow-band vegetation indices for prediction of crop chlorophyll content for application to precision agriculture. Remote Sens. Environ. 81 (2/3), 416–426. https://doi.org/10.1016/s0034-4257(02)00018-4.

Hatfield, J.L., Boote, K.J., Kimball, B.A., Ziska, L.H., Izaurralde, R.C., et al., 2011. Climate 2oduction. Agron. J. 103 (2), 351–370. https://doi.org/10.2134/agronj2010.0303.

Hsu, P.-K., Dubeaux, G., Takahashi, Y., Schroeder, J.I., 2021. Signaling mechanisms in abscisic acid-mediated stomatal closure. Plant J. 105 (2), 307–321. https://doi.org/10.1111/tpj.15067.

Jumrani, K., Bhatia, V.S., 2018. Impact of combined stress of high temperature and water deficit on growth and seed yield of soybean. Physiol. Mol. Biol. Plants 24 (1), 37–50. https://doi.org/10.1007/s12298-017-0480-5.

Jurik, T.W., 1986. Temporal and spatial patterns of specific leaf weight in successional northern hardwood tree species. Am. J. Bot. 73 (8), 1083–1092.

Kanai, M., Yamada, T., Hayashi, M., Mano, S., Nishimura, M., 2019. Soybean (*Glycine max* L.) triacylglycerol lipase GmSDP1 regulates the quality and quantity of seed oil. Sci. Rep. 9 (1), 8924. https://doi.org/10.1038/s41598-019-45331-8.

Kataria, S., Jajoo, A., Guruprasad, K.N., 2014. Impact of increasing Ultraviolet-B (UV-B) radiation on photosynthetic processes. J. Photochem. Photobiol. B 137, 55–66. https://doi.org/10.1016/j.jphotobiol.2014.02.004.

Koti, S., Reddy, K.R., Reddy, V.R., Kakani, V.G., Zhao, D., 2005. Interactive effects of carbon dioxide, temperature, and ultraviolet-B radiation on soybean (*Glycine max* L.) flower and pollen morphology, pollen production, germination, and tube lengths. J. Exp. Bot. 56 (412), 725–736. https://doi.org/10.1093/jxb/eri044.

Krishnan, H.B., Kim, W.-S., Oehrle, N.W., Smith, J.R., Gillman, J.D., 2020. Effect of heat stress on seed protein composition and ultrastructure of protein storage vacuoles in the cotyledonary parenchyma cells of soybean genotypes that are either tolerant or sensitive to elevated temperatures. Int. J. Mol. Sci. 21 (13), 4775. https://doi.org/10.3390/ijms21134775.

Kumar, N., Mishra, B.K., Liu, J., Mohan, B., Thingujam, D., et al., 2023. Network biology analyses and dynamic modeling of gene regulatory networks under drought stress reveal major transcriptional regulators in arabidopsis. Int. J. Mol. Sci. 24 (8), 7349. https://doi.org/10.3390/ijms24087349.

Lawas, L.M.F., Shi, W., Yoshimoto, M., Hasegawa, T., Hincha, D.K., et al., 2018. Combined drought and heat stress impact during flowering and grain filling in contrasting rice cultivars grown under field conditions. Field Crops Res 229, 66–77. https://doi.org/10.1016/j.fcr.2018.09.009.

Lenth, R.V., 2016. Least-Squares Means: The R package lsmeans. J. Stat. Softw. 69 (1), 33. https://doi.org/10.18637/jss.v069.i01.

Li, M.-W., Xin, D., Gao, Y., Li, K.-P., Fan, K., et al., 2017. Using genomic information to improve soybean adaptability to climate change. J. Exp. Bot. 68 (8), 1823–1834. https://doi.org/10.1093/jxb/erw348.

Lima, M.C.F., Krus, A., Valero, C., Barrientos, A., del Cerro, J., et al., 2020. Monitoring plant status and fertilization strategy through multispectral images. Sensors 20 (2), 435. https://doi.org/10.3390/s20020435.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25 (4), 402–408. https://doi.org/10.1006/meth.2001.1262.

Matiu, M., Ankerst, D.P., Menzel, A., 2017. Interactions between temperature and drought in global and regional crop yield variability during 1961-2014. Plos One 12 (5), e0178339. https://doi.org/10.1371/journal.pone.0178339.

Mendiburu, F. de, and M. Yaseen. 2020. Statistical procedures for agricultural research. R package version 1.4.0. https://myaseen208.github.io/agricolae/https://cran.r-project.org/package=agricolae.

Mittler, R., 2006. Abiotic stress, the field environment and stress combination. Trends Plant Sci. 11 (1), 15–19. https://doi.org/10.1016/j.tplants.2005.11.002.

Mourtzinis, S., Gaspar, A.P., Naeve, S.L., Conley, S.P., 2017. Planting date, maturity, and temperature effects on soybean seed yield and composition. Agron. J. 109 (5), 2040–2049. https://doi.org/10.2134/agronj2017.05.0247.

Msanne, J., Lin, J., Stone, J.M., Awada, T., 2011. Characterization of abiotic stress-responsive *Arabidopsis thaliana* RD29A and RD29B genes and evaluation of transgenes. Planta 234 (1), 97–107. https://doi.org/10.1007/s00425-011-1387-y.

Ney, B., Duthion, C., Ture, O., 1994. Phenological responses of pea to water stress during reproductive development. Crop Sci. 34, 141–146.

Onat, B., Bakal, H., Gulluoglu, L., Arioglu, H., 2017. The effects of high temperature at the growing period on yield and yield components of soybean [*Glycine max* (l.) merr] varieties. Turk. J. Field Crop. 22 (2), 178–186. https://doi.org/10.17557/tjfc.356210.

Pandey, P., Ramegowda, V., Senthil-Kumar, M., 2015. Shared and unique responses of plants to multiple individual stresses and stress combinations: physiological and molecular mechanisms. Front. Plant Sci. 6, 723. https://doi.org/10.3389/fpls.2015.00723.

Poudel, S., Adhikari, B., Dhillon, J., Reddy, K.R., Stetina, S.R., et al., 2023a. Quantifying the physiological, yield, and quality plasticity of Southern USA soybeans under heat stress. Plant Stress 9, 100195. https://doi.org/10.1016/j.stress.2023.100195.

Poudel, S., Vennam, R.R., Shrestha, A., Reddy, K.R., Wijewardane, N.K., et al., 2023b. Resilience of soybean cultivars to drought stress during flowering and early seed-setting stages. Sci. Rep. 13 (1), 1277. https://doi.org/10.1038/s41598-023-28354-0.

Pradhan, G.P., Prasad, P.V.V., Fritz, A.K., Kirkham, M.B., Gill, B.S., et al., 2012. Effects of drought and high temperature stress on synthetic hexaploid wheat. Funct. Plant Biol. 39 (3), 190–198. https://doi.org/10.1071/FP11245.

Prasad, P.V.V., Bheemanahalli, R., Jagadish, S.V.K., 2017. Field crops and the fear of heat stress—Opportunities, challenges and future directions. Field Crop. Res. 200, 114–121. https://doi.org/10.1016/j.fcr.2016.09.024.

Prasch, C.M., Sonnewald, U., 2013. Simultaneous application of heat, drought, and virus to arabidopsis plants reveals significant shifts in signaling networks. Plant Physiol. 162 (4), 1849–1866. https://doi.org/10.1104/pp.113.221044.

Puteh, A., M. Thuzar, M.M.A. Mondal, A. Abdullah, and M. Halim. 2013. Soybean [*Glycine max* (L.) Merrill] seed yield response to high temperature stress during reproductive growth stages. Aust. J. Crop Sci. https://www.semanticscholar.org/paper/Soybean-%5BGlycine-max-(L.)-Merrill%5D-seed-yield-to-Puteh-Thuzar/37a98e2b3c1ea83691864cb5e6449abb919bd19d (accessed 18 December 2023).

Rizhsky, L., Liang, H., Mittler, R., 2002. The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiol. 130 (3), 1143–1151. https://doi.org/10.1104/pp.006858.

Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davletova, S., et al., 2004. When defense pathways collide. the response of arabidopsis to a combination of drought and heat stress. Plant Physiol. 134 (4), 1683–1696. https://doi.org/10.1104/pp.103.033431.

Schauberger, B., Archontoulis, S., Arneth, A., Balkovic, J., Ciais, P., et al., 2017. Consistent negative response of US crops to high temperatures in observations and crop models. Nat. Commun. 8 (1), 13931 https://doi.org/10.1038/ncomms13931.

Sinha, R., Induri, S.P., Peláez-Vico, M.Á., Tukuli, A., Shostak, B., et al., 2023. The transcriptome of soybean reproductive tissues subjected to water deficit, heat stress, and a combination of water deficit and heat stress. Plant J. 116 (4), 1064–1080. https://doi.org/10.1111/tpj.16222.

Sinha, R., Zandalinas, S.I., Fichman, Y., Sen, S., Zeng, S., et al., 2022. Differential regulation of flower transpiration during abiotic stress in annual plants. N. Phytol. 235 (2), 611–629. https://doi.org/10.1111/nph.18162.

Steele, M.R., Gitelson, A.A., Rundquist, D.C., 2008. A comparison of two techniques for nondestructive measurement of chlorophyll content in grapevine leaves. agj2agronj20070254n Agron. J. 100 (3). https://doi.org/10.2134/agronj2007.0254N.

Thompson, C.N., Guo, W., Sharma, B., Ritchie, G.L., 2019. Using normalized difference red edge index to assess maturity in cotton. Crop Sci. 59 (5), 2167–2177. https://doi.org/10.2135/cropsci2019.04.0227.

USDA Drought Monitor. 2023. Current Map | U.S. Drought Monitor. https://droughtmonitor.unl.edu/ (accessed 28 December 2023).

USDA ERS. 2023. Warming temperatures in U.S. Corn Belt expected to continue into next decade. http://199.135.94.241/data-products/chart-gallery/gallery/chart-detail/?chartId=108037 (accessed 28 December 2023).

Vennam, R.R., Poudel, S., Ramamoorthy, P., Samiappan, S., Reddy, K.R., et al., 2023a. Impact of soil moisture stress during the silk emergence and grain-filling in maize. Physiol. Plant. 175 (5), e14029 https://doi.org/10.1111/ppl.14029.

Vennam, R.R., Ramamoorthy, P., Poudel, S., Reddy, K.R., Henry, W.B., et al., 2023b. Developing functional relationships between soil moisture content and corn early-season physiology, growth, and development. Plants 12 (13), 2471. https://doi.org/10.3390/plants12132471.

Vincini, M., Frazzi, E., D'Alessio, P., 2008. A broad-band leaf chlorophyll vegetation index at the canopy scale. Precis. Agric. 9 (5), 303–319. https://doi.org/10.1007/s11119-008-9075-z.

Walter-Shea, E.A., Norman, J.M., Blad, B.L., Robinson, B.F., 1991. Leaf reflectance and transmittance in soybean and corn. Agron. J. 83 (3), 631–636. https://doi.org/10.2134/agronj1991.00021962008300030026x.

Wang, D., Heckathorn, S.A., Barua, D., Joshi, P., Hamilton, E.W., et al., 2008. Effects of elevated CO_2 on the tolerance of photosynthesis to acute heat stress in C_3, C_4, and CAM species. Am. J. Bot. 95 (2), 165–176. https://doi.org/10.3732/ajb.95.2.165.

Wang, L., Liu, L., Ma, Y., Li, S., Dong, S., et al., 2018. Transcriptome analysis characterized the gene expression patterns responded to combined drought and heat stresses in soybean. Comput. Biol. Chem. 77, 413–429. https://doi.org/10.1016/j.compbiolchem.2018.09.012.

Xu, C., Xia, Z., Huang, Z., Xia, C., Huang, J., Zha, M., Wang, S., Imran, S., Casteel., Jiang, Y., Zhang, C., 2019. Understanding the physiological and transcriptional mechanism of reproductive stage soybean in response to heat stress. Crop Breed. Genet. Genom. 2 (1), e200004 https://doi.org/10.20900/cbgg20200004.

Yu, Q., Hua, X., Yao, H., Zhang, Q., He, J., et al., 2021b. Abscisic acid receptors are involves in the Jasmonate signaling in Arabidopsis. Plant Signal. Behav. 16 (10), 1948243. https://doi.org/10.1080/15592324.2021.1948243.

Yu, C., Miao, R., Khanna, M., 2021a. Maladaptation of U.S. corn and soybeans to a changing climate. Sci. Rep. 11 (1), 12351 https://doi.org/10.1038/s41598-021-91192-5.

Zandalinas, S.I., Fritschi, F.B., Mittler, R., 2020. Signal transduction networks during stress combination. J. Exp. Bot. 71 (5), 1734–1741. https://doi.org/10.1093/jxb/erz486.

Zandalinas, S.I., Mittler, R., Balfagón, D., Arbona, V., Gómez-Cadenas, A., 2018. Plant adaptations to the combination of drought and high temperatures. Physiol. Plant. 162 (1), 2–12. https://doi.org/10.1111/ppl.12540.

Zhang, B., Feng, G., Read, J.J., Kong, X., Ouyang, Y., et al., 2016. Simulating soybean productivity under rainfed conditions for major soil types using APEX model in East Central Mississippi. Agric. Water Manag. 177, 379–391. https://doi.org/10.1016/j.agwat.2016.08.022.

Zhang, Q., Li, Q., Zhang, G., 2012. Rapid determination of leaf water content using vis/nir spectroscopy analysis with wavelength selection. J. Spectrosc. 27, 93–105. https://doi.org/10.1155/2012/276795.

Zhang, H., Sonnewald, U., 2017. Differences and commonalities of plant responses to single and combined stresses. Plant J. 90 (5), 839–855. https://doi.org/10.1111/tpj.13557.

Zhang, H., Zhu, J., Gong, Z., Zhu, J.-K., 2022. Abiotic stress responses in plants. Nat. Rev. Genet. 23 (2), 104–119. https://doi.org/10.1038/s41576-021-00413-0.

Zhu, Y.-Y., Thakur, K., Feng, J.-Y., Cai, J.-S., Zhang, J.-G., et al., 2020. B-vitamin enriched fermented soymilk: a novel strategy for soy-based functional foods development. Trends Food Sci. Technol. 105, 43–55. https://doi.org/10.1016/j.tifs.2020.08.019.

Zinta, G., AbdElgawad, H., Peshev, D., Weedon, J.T., Van den Ende, W., et al., 2018. Dynamics of metabolic responses to periods of combined heat and drought in *Arabidopsis thaliana* under ambient and elevated atmospheric CO2. J. Exp. Bot. 69 (8), 2159–2170. https://doi.org/10.1093/jxb/ery055.