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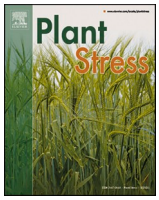

# Exploring the synergistic effects of drought and heat stress on chickpea seed development: Insights into nutritional quality and seed yield

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ABSTRACT

Growing chickpea (*Cicer arietinum* L.) faces significant challenges due to rising temperatures and drought stress, particularly during the reproductive and seed-filling phases. This study investigated the single and joint impacts of drought and heat stress on seed development, focusing on the responses of drought-tolerant (DT) and drought-sensitive (DS) chickpea genotypes. Initially raised in an outdoor environment (mean day and night temperature of 27 and 16±1 °C, respectively, light intensity of 1230–1440 μmol $m^{-2}$ $s^{-1}$, relative humidity of 70/43 %) until seed filling (around 110–113 days after sowing) commenced. The plants were subsequently exposed to single or combined heat and drought stress under controlled conditions until maturity. Control pots were maintained at day and night temperature of 25 and 15 °C, respectively with 500 μmol $m^{-2}$ $s^{-1}$ light, 60–65 % RH, and regular irrigation, and drought-stressed pots were kept at 50 % field capacity under the same conditions of light and humidity. Heat stress in pots was gradually increased to 32(day)/20 °C (night) under regular irrigation, while combined stress pots experienced both drought (50 % field capacity) and heat stress conditions 32(day)/20 °C (night) under the same light and humidity conditions with irrigation. All stress treatments adversely affected cell membranes, photosynthesis, and water regulation, with more pronounced effects under combined stress. While heat stress increased stomatal conductance, drought and combined stress significantly reduced it. Seed filling rate and duration decreased under all stress conditions, especially combined stress. The stresses in combination severely reduced seed weight and pod numbers compared to individual stresses. Enzyme activities involved in starch and sucrose synthesis and hydrolysis substantially decreased under the combined stress. Seed composition elements (starch, storage proteins, sugars, fat, crude fiber, and ash) exhibited significant reductions across all stress treatments, particularly for the combined stress. Thus, under combined stresses, starch, proteins, and soulube sugars were markedly decreased to 13–20 %, 6.4–12.4 %, and 3–5 % in seeds, compared to 37–39 %, 21–24 %, and 6 % in control seeds. The DT genotype outperformed the DS genotype for all traits under individual and combined stress conditions. Principal component analysis revealed a complex interplay among various physiological responses (membrane damage, chlorophyll, chlorophyll fluorescence, relative leaf water content, and stomatal conductance), seed yield, and seed composition under the combined stress. This study highlighted that combined heat and drought stress severely impacted chickpea yield and nutritional traits, such as seed starch and protein content, compared to individual stresses underscoring the need to develop cultivars tolerant to this stress combination.

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## 1. Introduction

Environmental challenges such as high temperature and water deficits significantly limit crop growth and productivity (Barnabas et al., 2008). While the separate impacts of drought and heat stress on crops have been studied extensively, there is a relative scarcity of experiments investigating the combined effects of these stressors despite their interconnectedness and adverse effects on crop development and productivity (Nankishore and Farrell, 2016; Lamaoui et al., 2018; Hein et al., 2021; Ru et al., 2022; Yadav et al., 2022). Global climate change exacerbates these concerns, manifesting in increasing temperatures, altered precipitation patterns, and increased drought occurrences in semi-arid and arid regions, ultimately jeopardising global crop productivity (Singh et al., 2023). Projections indicate a continued rise in the occurrence of coupled high temperatures and drought (IPCC, 2014), highlighting the urgency to investigate this stress combination for enhancing crop tolerance in the face of evolving climate conditions (Zandalinas et al., 2017). Despite studies on the combined influence of heat and drought stress in certain crops like maize (Zea mays L.) (Cairns et al., 2013), wheat (Tritium aestivum L.) (Wardlaw, 2002), groundnut (Arachis hypogaea L.) (Hamidou et al., 2012), canola (Brassica napus L.), and lentil (Lens culinaris Medikus), there is a noticeable gap in similar investigations for chickpea (Cicer arietinum L.) (Awasthi et al., 2014).

The combination of heat and drought stress exerts a more profound effect on plant growth and productivity than their separate effects (Barnabas et al., 2008; De Boeck et al., 2016; Zandalinas et al., 2016). Reproductive stages of plants, especially during flowering and anthesis, are particularly susceptible to these stresses (Barnabas et al., 2008; Devi et al., 2022), resulting in fertilisation failure due to compromised functionality of pollen and ovule, disrupted pollen development, and pollen sterility (Prasad et al., 2008). The combination of heat and drought stress severely influences the reproductive events in various crops, including legumes like groundnut (Prasad et al., 2000; Sadras et al., 2013; Yadav et al., 2022) and lentil (Sita et al., 2018) and cereals like wheat and maize (Barnabas et al., 2008; Prasad et al., 2011). Despite the known reductions in leaf area, photosynthesis, stomatal conductance, and water use efficiency under the combination of both stresses in cereals (Shah and Paulsen, 2003), there is limited information on the responses of legumes to this combined stress at physiological and biochemical levels. Hence, further study is needed to comprehend the stress tolerance mechanisms in leguminous crops.

Seed development is a critical stage of growth for grain crops, involving processes that facilitate the transport of resources from leaves and the formation of macromolecules in seeds (Triboi et al., 2003; Behboudian et al., 2001; Ahmadi and Baker, 2001). The occurrence of drought in conjunction with heat stress at the time of seed filling decreases yield in various legumes (Canci and Toker, 2009; Sehgal et al., 2018; Kumari et al., 2021), cereals (Ben Mariem et al., 2021), and other crops like safflower (*Carthamus tinctorius* L.) (Houshmand et al., 2021), cotton (*Gossypium hirsutum* L.) (Hu et al., 2023), and canola (*Brassica napus* L.) (Secchi et al., 2023).

Sucrose metabolism plays a critical role during seed filling by maintaining a balance between hexose and sucrose, which is essential for regulating vital phases of seed development (Weschke et al., 2000). Drought stress decreased the activities of acid invertases in maize, affecting grain development (Zinselmeier et al., 1999; Weschke et al., 2000; Andersen et al., 2002). Drought stress also disrupts metabolic pools downstream of sucrose during the synthesis of starch, impairing grain filling (Zinselmeier et al., 1999). Consequently, combined heat and drought stress may exacerbate the transfer and utilisation of assimilates essential for successful seed filling, but limited information is available on this aspect, especially in legumes.

Chickpea, a prominent legume crop, thrives best within a temperature range of 20–28 °C during its reproductive stage. Cultivated as a rainfed crop in northern India during the winter months from November to April (Devasirvatham et al., 2013), elevated temperatures (>32 °C) and drought during the crucial seed-filling phase (mid-February to April) present significant challenges to chickpea growers (Kaushal et al., 2013).

Chickpea is highly susceptible to the combined effects of heat and drought stress, and studies have shown that these two abiotic stresses can have a significant additive impact on chickpea's growth, yield and physiological responses (Awasthi et al., 2014, 2017; Benali et al., 2023), which need to be investigated in detail. Considering this, the present study was planned to evaluate the concurrent impact of drought and heat stress during seed filling on yield and nutritional traits in chickpea genotypes exhibiting varying sensitivity to drought stress. It was hypothesised that the degree of drought tolerance in chickpea genotypes would influence their ability to cope with the combined stresses of drought and heat, leading to differences in yield and nutritional quality under these conditions. The outcomes of this research seek to provide valuable insights into the response of chickpeas to a combination of heat and drought stress, facilitating the development of approaches to mitigate the adverse effects of climate change on agriculture.

## 2. Methods

### 2.1. Plant cultivation

The study was conducted at Panjab University, Chandigarh, India (30.7333° N, 76.7794° E), focusing on one drought-tolerant (ICC8950; yellow-brown seeded; Desi type) and one drought-sensitive (ICC3776; black-seeded; Desi type) chickpea genotype, sourced from the Indian Institute of Pulses Research, India. Plants were grown in earthenware pots (15 cm diameter, 20 cm height; 8 kg soil capapcity) filled with air-dried soil, sand, and farmyard manure in a 2:1:1 (v/v) ratio. The soil, classified as loam with pH 7.3, contained 51 kg $ha^{-1}$ available nitrogen (N), 39 kg $ha^{-1}$ available phosphorus (P), and 143 kg $ha^{-1}$ available potassium (K). *Rhizobium ciceri* (1.95 g $kg^{-1}$ seeds) was applied to the seeds at the recommended rate. Three seeds were sown per pot in early November 2020, and reduced to two plants per pot following germination. The plants were raised outdoors in a wired-protective enclosure (to prevent damage from birds and animals), exposed to natural environmental conditions (mean day and night temperature of 27 and 16±1 °C, respectively light intensity of 1230–1440 μmol $m^{-2}$ $s^{-1}$, relative humidity of 70 (day)/43 % (night) in a randomized block design (Fig. 1). The pots were moved regularly to avoid positional effects, maintaining this setup until the start of seed filling, around 110–113 days after sowing (DAS). Subsequently, the pots were transferred to various growth chambers exposure to one of four stress treatments at the beginning of seed filling for 30 days until maturity (140–143 DAS). Each treatment comprised 30 pots—ten pots replicated three times.

### 2.2. Treatments

In the control treatment, pots were maintained at a day and night temperature of 25 and 15 °C, respectively, with a with a light intensity of ~500 μmol $m^{-2}$ $s^{-1}$, RH of 60–65 %, and the pots were irrigated twice daily to prevent dehydration. For the heat stress treatment, the pots were kept at a constant temperature of 32 °C during the day and 20 °C at night, a light intensity of 500 μmol $m^{-2}$ $s^{-1}$, and RH of 60–65 %. The temperature of the chamber was increased gradually, starting at a mean day and night temperature of 28 and 17 °C, respectively and increasing by 1 °C per day to achieve the final temperature. Adequate irrigation was provided to prevent soil water stress and maintain hydration. For the drought stress treatment, pots received approximately half the amount of water typically needed to maintain soil moisture at field capacity. Soil moisture levels at 10–15 cm depth were monitored regularly by means of a soil moisture sensor probe (Spectrum Technologies, USA). The other environmental conditions included maintaing day and night temperature of 25 and 15 °C, respectively, a light intensity of ~500 μmol

$m^{-2}$ $s^{-1}$, and RH of 60–65 %. For the combined stress treatment, pots were subjected to drought stress at 110–113 DAS by restricting irrigation and keeping the soil at half of its maximum water retention capacity. Additionally, the plants were exposed to a controlled heat stress environment, as described for the heat stress treatment. In each treatment, there were 10 pots, each containing 2 plants in a pot across the 3 replicates, resulting in a total of 60 plants per treatment.

### 2.3. Evaluation of stress-induced injury

Following a 10-day period in a controlled environment, leaves were collected at 11:00AM from the second and third branches at the topmost parts of both the control and stressed plants.Nine plants per treatment (three per replicate) were used to assess various leaf traits, including relative electrolyte leakage (EL), leaf water content (RLWC), PSII function, chlorophyll levels, and stomatal conductance.

For measuring the EL (%), fresh leaf samples weighing 100 mg each, taken from the upper branches, were thoroughly cleansed with deionized water to eliminate any electrolytes on the leaf surface. Subsequently, they were placed into sealed glass vials with deionized water (10 mL), followed by incubation on a rotary shaker at 25 °C for 24 h. After the incubation period, the electrical conductivity (EC) of the solution (L1) was assessed with a conductivity meter. Subsequently, these vials were warmed for 20 min at 120 °C in a water bath, with the final EC (L2) determined after reaching equilibrium at 25 °C (Lutts, 1996).

Segments of leaves (100 mg each) were submerged in distilled water within a Petri dish for a duration of 2 h. Following removal and surface drying using blotting sheets, the weight of the turgid leaves (TW) was

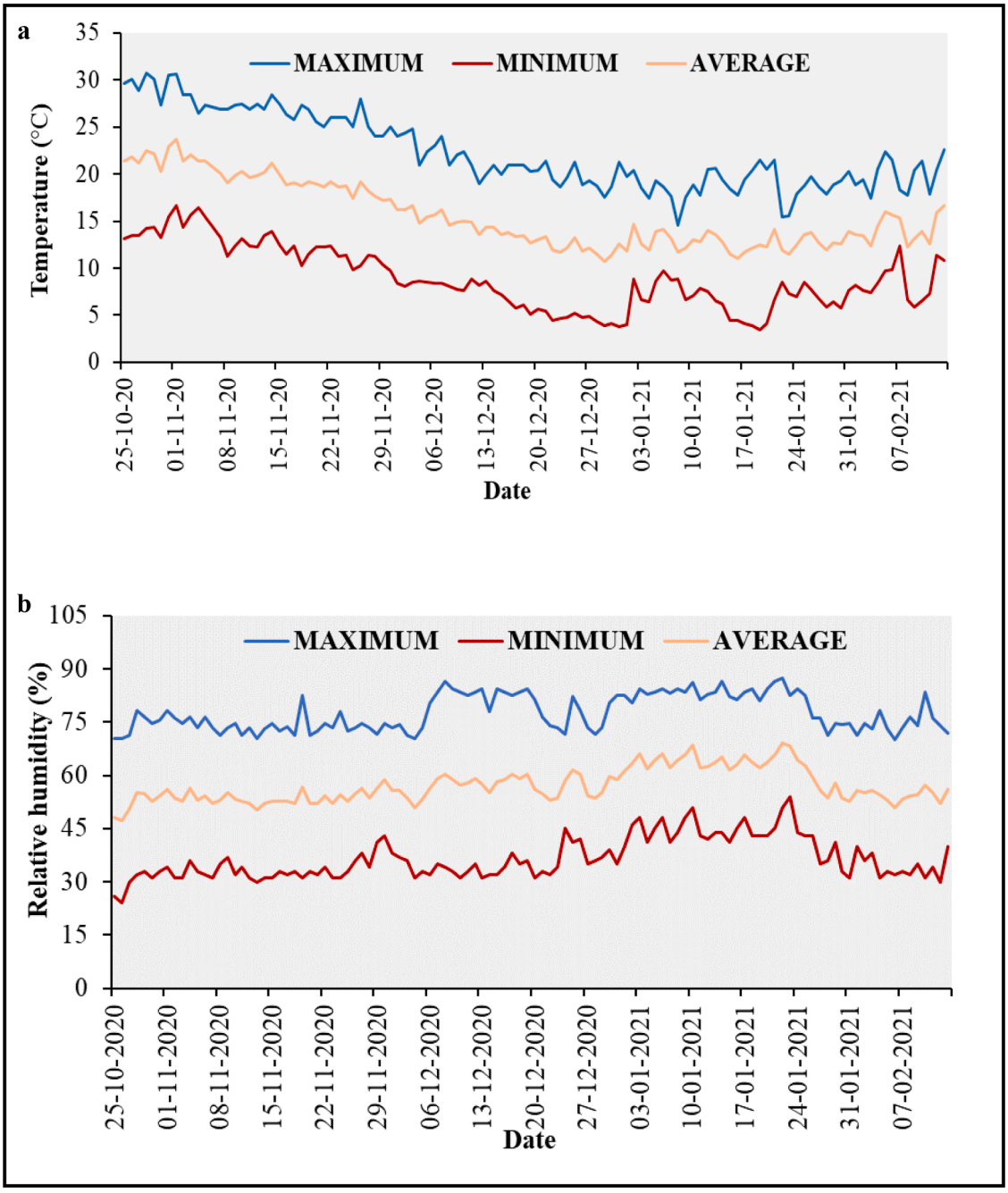


**Fig. 1.** Maximum and minimum temperatures (a) and relative humidity (RH%) (b) in an outdoor environment at Panjab University, Chandigarh, India during cultivation of chickpea in pots until the start of seed filling (110–113 days after sowing).

recorded. Subsequently, the tissue was dried in an oven at 110 °C for 24 h, and the dry weight (DW) was measured. RLWC (%) was measured using the formula: RLWC = (FW −DW) / (TW −DW) × 100, and expressed as % (Barrs and Weatherley, 1962).

Chlorophyll (mg $g^{-1}$ dw) was extracted from fresh leaf samples from the upper branches using the method described by Arnon (1949). The absorbance of the extract was measured with a spectrophotometer at 663 and 645 nm.

Photochemical efficiency (Fv/Fm ratio) was evaluated by examining chlorophyll fluorescence by means of a dark-adapted test with an "OS1-FL modulated chlorophyll fluorometer (Opti-Sciences,USA)". The "Fv/Fm value", suggestive of the highest "quantum yield" in "PSII photochemistry" and a measure of PSII activity, was recorded following the methodology outlined in Awasthi et al. (2014).

The conductance of stomata (expressed as mmol $m^{-2}$ $s^{-1}$) in fully developed leaves taken from the top branches was evaluated by means of a portable leaf porometer (Decagon Devices, USA), following the methodology in Awasthi et al. (2014).

### 2.4. Enzyme assays

The activities of sucrose synthase (EC 2.4.1.13; μmol $min^{-1}$ protein), soluble starch synthase (EC 2.4.1.21; μmol $min^{-1}$ protein), and acid invertase (EC 3.2.1.26; μmol $min^{-1}$ protein) were determined in freshly harvested seeds at physiological maturity. Seeds were harvested from pods on the top branches of plants grown in a controlled and stressful environment. Extractions were performed on seed samples in an ice-cooled HEPES/KOH buffer (200 mM; pH 7.8). The buffer also included 10 mM dithiothreitol, 3 mM magnesium acetate, 1 % (w/v) polyvinylpyrrolidone, and 3 mM EDTA $Na_22H_2O$. Centrifugation of the homogenate was carried out at 10,000 *g* for 20 min at 4 °C, with the resulting supernatant serving as the source of enzymes and proteins. The supernatant was filtered through pre-equilibrated "Sephadex G-25 columns" (Sigma, USA) to facilitate desalting. The desalting process utilised a buffer solution comprising "HEPES-NaOH (20 mM; pH 7.5)", 0.01 % 2-mercaptoethanol, 0.25 mM $MgCl_2$, 0.05 % BSA, and 1 mM EDTA, all kept at 4 °C. The desalted extract was measured using a method described by Racker (1962). Enzyme activities were determined at 25 °C following the procedures outlined by Xu et al. (1996) and Sung et al. (1989).

### 2.5. Seed reserves

Seed reserves were evaluated by examining mature seeds collected at 143–146 DAS for control plants and 132–135 DAS for stressed plants. The soluble sugars (glucose, fructose, and sucrose) and starch were obtained involving ethanol 95 % (v/v) and perchloric acid 30 % (v/v), sequentially. Phenol–sulfuric acid method was used to quantify these molecules (Dubois et al. 1956).

For soluble sugars analysis (expressed as g 100 $g^{-1}$), 100 mg of dried powdered tissue was extracted and then heated to 90 °C in 3 mL of 80 % ethanol. After centrifuging the mixture for 15 min at 10,000 *g*, the supernatant was recovered. The procedure of ethanol extraction and centrifugation was performed thrice, and the collected supernatant was pooled.

After washing three times, the remaining solid residue was used for analysis of starch (expressed as g 100 $g^{-1}$). The overall pooled supernatant was brought to a total volume of 9 mL using 80 % ethanol. To eliminate interfering compounds, 60 mg of activated charcoal (EC 231–153–3) was added. The mixture was then vortexed and allowed to settle for 5 min at room temperature. Following a second vortexing, the mixture underwent centrifugation at 10,000 g for 30 min.

For the soluble sugars analysis, 3 mL of pooled supernatant was added to a glass test tube, and ethanol was allowed to evaporate for 60 min. A working solution containing HEPES buffer (50 mM; pH 7.2), 20 mM ATP, and 20 mM NAD dissolved in double-distilled water was added to each sample. Afterward, 200 μL of the glucose assay reagent (Glucose Assay Kit, Sigma, USA) was added to each sample, and the mixture was kept for incubation for 15 min in the dark at room temperature. Absorbance changes resulting from the conversion of glucose-6-phosphate to 6-phosphogluconate were measured at 340 nm using a UV–Vis spectrophotometer. For fructose analysis, an additional 20 μL of phosphoglucose isomerase (0.25 units, Sigma P-9544) was added; subsequently, the mixture was incubated for 15 min at room temperature, and absorbance was determined at 340 nm. For sucrose analysis, 40 μL of invertase (Sigma I614504) was included in each well; subsequently, the mixture was kept for 60 min at room temperature, and absorbance was measured at 340 nm. Carbohydrate concentrations were determined by means of a standard curve of glucose concentrations, and the results were expressed as μmol $g^{-1}$ dw (Zhao et al., 2010).

A conventional AOAC method was employed to analyse crude protein and ash (measured via "micro-Kjeldahl, $N \times 6.25''$), fibre (g 100 $g^{-1}$), fat (g 100 $g^{-1}$), and ash contents. The extraction of different protein fractions (expressed as g 100 $g^{-1}$) such as albumins, glutelins, prolamins, and globulins was carried out serially from the seeds following the protocol outlined in Triboi et al. (2000). Wholemeal flour was obtained by grinding the seeds. Each extraction stage involved continuous stirring for 60 min, followed by centrifugation at 8000 *g* for 30 min to separate soluble and insoluble fractions. Albumin and globulins were separated individually at 4 °C using 25 mL of 0.05 M sodium phosphate buffer (pH 7.8) and 25 mL of 0.05 M NaCl, respectively. The residual pellet from the preceding extraction phase underwent further extraction using 25 mL of a mixture containing "2 % (v/v) Triton X-114, 0.1 M sodium chloride, and 0.05 M sodium phosphate buffer (pH 7.8)." Prolamin was obtained from the pellet at 20 °C using ethanol, and glutelins were extracted at 20 °C using 25 mL of a solution comprising sodium dodecyl sulphate, 2 % "2-mercaptoethanol, and 0.05 M tetraborate buffer (pH 8.5)". The protein content within each fraction was quantified as per the methodology in Lowry et al. (1951).

### 2.6. Seed growth rate and seed filling duration

Nine plant treatment$^{-1}$ (three per replicate) were chosen to evaluate seed filling rate (mg $day^{-1}$) and seed filling duration (days). Five pods $plant^{-1}$ were marked at the onset of pod filling when the pod size was approximately 1 cm and monitored until physiological maturity (132–136 DAS for control plants and 122–124 DAS for stressed plants). The weight of dried seeds was measured twice: one week after the pods began to fill and again when they were fully physiologically mature. The seeds were subjected to drying in an oven at 45 °C for five days before being weighed. The duration required for each labeled pod to complete seed filling was recorded.

### 2.7. Yield traits

Seed weight (g $plant^{-1}$), both per plant and individual, was measured from nine plants (three per replicate) of each genotype.

### 2.8. Statistical analysis

Statistical analysis was performed on the collected data, calculating means and standard errors. A two-way ANOVA was performed, with least significant differences computed for traits with significant differences ($P < 0.05$). Principal component analysis (PCA) was done by means of R-statistical software.

## 3. Results

### 3.1. Leaf traits

Control plants had RLWC values ranging from 80 to 82 %. However, individual drought and heat stresses significantly decreased RLWC to

56–62 % and 64–72 %, respectively, while the combined stress further decreased RLWC to 42–61 % (Fig. 2b). In all stress treatments, the drought-tolerant (DT) genotype exhibited markedly higher relative leaf water content (RLWC) compared to the drought-sensitive (DS) genotype.

Membrane damage (EL%) in the leaves of control plants ranged from 9.5 to 11.4 %, increasing to 16–21 % under drought stress, 13–17 % under heat stress, and 22–27 % under combined stress (Fig. 2a). Across all stress treatments, the DS genotype exhibited notably greater membrane damage compared to the DT genotype.

Leaf chlorophyll concentration significantly decreased under drought stress (42–58 %), heat stress (29–46 %), and the combined stress (54–67 %) relative to the control. The DT genotype had 16 %, 17 %, and 13 % higher chlorophyll contents than the DS genotype under drought, heat, and their combination, respectively (Fig. 3b).

Photosystem II (PSII) function decreased more significantly in plants exposed to drought stress (16–22 %) than heat stress (10–15 %) relative to the control, with a more pronounced reduction under the combined stress (25–47 %) (Fig. 3a).The DT genotype had significantly higher PSII activity than the DS genotype across all stress treatments.

Stomatal conductance showed an increase in high-temperature-stressed chickpea plants but a decrease in water-stressed plants. The DT genotype had 24 %, 42 %, and 45 % higher stomatal conductance than the DS genotype under drought, heat, and a combination of stresses, respectively (Fig. 2c).

Correlation coeffcients of various traits with yield traits are shown in Table 2.

### 3.2. Yield traits

The seed-filling period decreased by 5.3–10 days under heat stress, 7–11 days under drought stress, and 10–15 days under the combination of stresses compared to the control (Table 1). Under drought, the DT genotype had a significantly longer seed-filling duration than the DS genotype (4 days), heat (5 days), and concurrent stress (4 days).

The seed-filling rate decreased by 15–17 % under heat stress, 23–31 % under drought stress, and 50–59 % under the stress combination compared to the control (Table 1). The DT genotype had significantly higher seed-filling rates than the DS genotype across all treatments.

Total seed weight per plant declined by 23–43 % under heat stress, 33–56 % under drought stress, and 57–66 % under the combined stress relative to the controls (Table 1).The DT genotype produced heavier seeds than the DS genotype across all stress treatments.

Pod number per plant declined by 26–35 % under drought stress, 14–19 % under heat stress, and by 51–88 %) under combined stress. The DT genotype produced significantly more pods per plant than the DS genotype across all stress treatments (Table 1).

### 3.3. Seed traits

#### 3.3.1. Seed components

The seed starch content of the control plants ranged from 37 to 39 %, decreasing to 27–31 % under high temperatures, 20–28 % under water deficits, and 13–20 % under combined stress (Fig. 4). The seed protein content of the control plants varied from 21 to 24 %, decreasing to

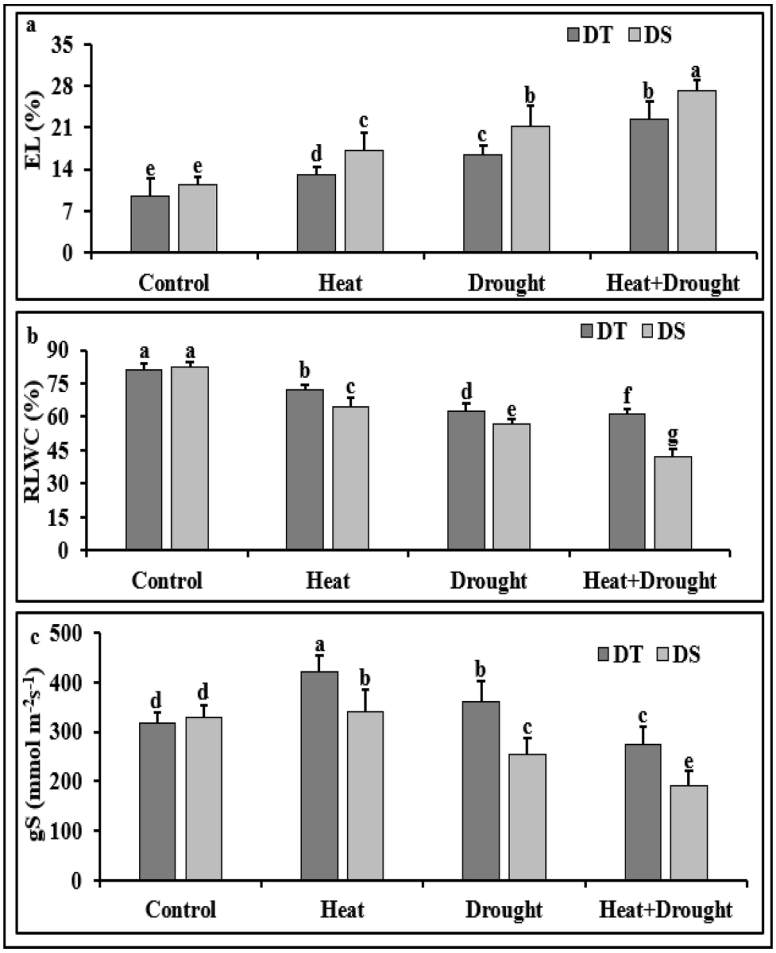


**Fig. 2.** Electrolyte leakage (%) (EL%) (a), relative leaf water content (RLWC) (b), and stomatal conductance (gS) (c) in drought-tolerant (DT) and drought-sensitive (DS) chickpea genotypes exposed to heat, drought, and combined heat and drought stress. LSD ($P < 0.05$): 3.8 (EL), 2.3 (RLWC), and gS (12.6). Different letters on bars indicate significant ($P < 0.05$) differences from each other. Values are mean ± *S*.E. ($n = 3$).

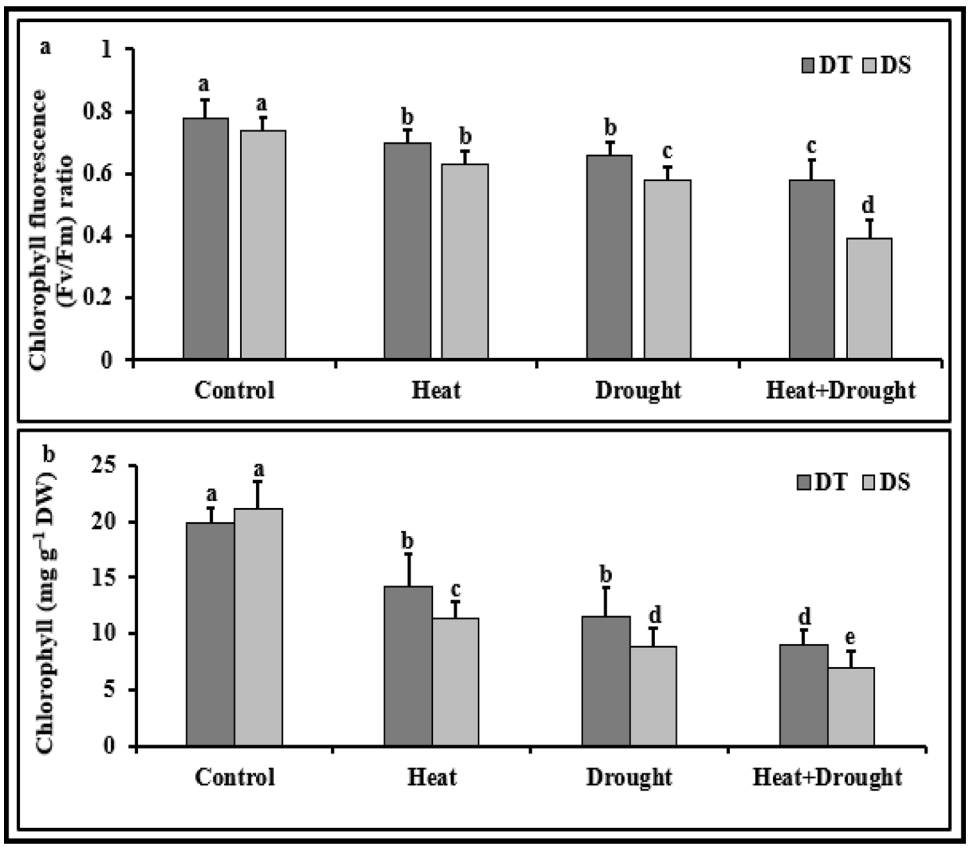


**Fig. 3.** Chlorophyll fluorescence(a) and chlorophyll concentration (b) in drought-tolerant (DT) and drought-sensitive (DS) chickpea genotypes exposed to heat, drought, and combined heat and drought stress. LSD ($P < 0.05$): 0.07 (chlorophyll fluorescence) and 1.5 (chlorophyll concentration). Different letters on bars indicate significant ($P < 0.05$) differences from each other.Values are mean ± *S*.E. ($n = 3$).

**Table 1**
Various seed traits in control and stressed chickpea plants. Values are means ± SE ($n = 3$) and LSD values ($P < 0.05$). Values with different lowercase letters within a row differ significantly.

| Trait | Control | | Heat stress | | Drought stress | | Heat + Drought stress | | LSD ($P < 0.05$): genotype × treatment interaction |
|---|---|---|---|---|---|---|---|---|---|
| | DT | DS | DT | DS | DT | DS | DT | DS | |
| Seed-filling duration (days) | 19.6 ± 1.3a | 21.3 ± 1.4b | 14.3 ± 1.3c | 11.3b ±1.2d | 12.4 ± 1.2e | 10.2 ± 1.2f | 9.5 ± 1.1f | 6.4 ± 1.1 g | 1.41 |
| Seed growth rate (mg day$^{-1}$) | 8.6 ± 0.53a | 7.7 ± 0.61b | 7.3 ± 0.62c | 6.4 ± 0.59d | 6.3 ± 0.60d | 5.3 ± 0.61e | 4.3 ± 0.58e | 3.2 ± 0.57f | 0.77 |
| Seed weight (g plant$^{-1}$) | 5.11 ±0.22a | 4.671 ±0.21b | 3.91 ±0.21b | 2.6 ± 0.23c | 3.41 ±0.22c | 2.02 ±0.20d | 2.21 ±0.18e | 1.56 ±0.17f | 0.28 |
| Individual seed weight (mg) | 143.2 ± 8.2a | 131.4 ± 7.2b | 127.3 ± 6.9c | 104.6 ± 7.5d | 107.6 ± 8.3e | 93.4 ± 7.4f | 89.7 ± 6.8 g | 63.4 ± 7.2 h | 1.8 |
| Pods plant$^{-1}$ | 14.3 ± 1.3a | 12.1 ± 1.1b | 12.1 ± 0.87b | 9.8 ± 0.67c | 10.5 ± 0.84c | 7.8 ± 0.66d | 6.9 ± 0.76d | 1.4 ± 0.38e | 1.5 |

14–20 % under heat stress, 11–17 % under drought stress, and 6.4–12.4 % under combined stress (Fig. 4). Soluble sugars in seeds increased slightly (~6 %) under individual heat or drought stress compared to controls but decreased to 3–5 % under combined stress (Fig. 4). Stress treatments significantly reduced fat, crude fibre, and ash contents, with the most pronounced effect under combined stress and smaller reductions in the DT genotype than the DS genotype (Fig. 4).

Seeds of control plants contained approximately 11 % albumins, 48–51 % globulins, 16–18 % glutelins, and 4 % prolamins (Fig. 5). The stress treatments led to a significant reduction in all seed storage proteins, with a more pronounced effect under drought stress compared to heat stress. The decrease was most severe under the stress combination, decreasing albumins to 3.5–6 %, globulins to 23–38 %, glutelins to 6–9.6 %, and prolamins to 0.6–2 %. Seeds of the DT genotype had markedly higher levels of seed proteins, especially globulins, than those of the DS genotype seeds across all stress treatments.

Seed sucrose concentration decreased by 30–42 % under drought stress, 19–20 % under heat stress, and 48–61 % under the joint treatment of stress relative to the control (Fig. 6). The DT genotype exhibited higher seed sucrose than the DS genotype across all stress treatments. Seed glucose and fructose concentrations considerably increased in individual drought and heat stress treatments but showed reductions with stress combinations, particularly in the DS genotype. The DT genotype had higher reducing sugars than the DS genotype across all stress treatments.

### 3.3.2. *Seed enzymes*

Soluble starch synthase activity declined by 24–43 % under drought stress, 10–31 % under heat stress, and 52–68 % under the combined stress compared to the control (Fig. 7b). Under the combined stress, the

**Table 2**
Correlation coefficients for various leaf and seed traits in chickpea plants grown under combined heat and drought stress.

| Traits | Pods plant$^{-1}$ | Single seed weight (SSW) | Total seed weight (TSW) | Seed filling duration (SFD) | Seed filling rate (SFR) |
|---|---|---|---|---|---|
| **Electrolyte leakage (EL%)** | –0.944** | –0.957** | –0.942** | –0.918** | –0.951** |
| **Stomatal conductance (gS)** | 0.972** | 0.971** | 0.855* | 0.935** | 0.705 ns |
| **Relative leaf water content (RLWC)** | 0.913** | 0.908** | 0.903** | 0.916** | 0.815* |
| **Photosynthetic efficiency (PS-II)** | 0.912*** | 0.908** | 0.914** | 0.913** | 0.861* |
| **Chlorophyll** | 0.914** | 0.915** | 0.910* | 0.918** | 0.873* |
| **Glucose** | 0.857** | 0.847* | 0.827* | 0.833* | 0.888* |
| **Fructose** | 0.823* | 0.834* | 0.873* | 0.870* | 0.880* |
| **Sucrose** | 0.871* | 0.883* | 0.860* | 0.865* | 0.811* |
| **Starch** | 0.871* | 0.979** | 0.986** | 0.847* | 0.892* |
| **Proteins** | 0.890* | 0.892* | 0.876* | 0.884* | 0.877* |
| **Soluble sugars** | 0.881* | 0.885* | 0.883* | 0.865* | 0.802* |
| **Prolamins** | 0.814* | 0.892* | 0.919** | 0.813* | 0.835* |
| **Glutelins** | 0.883* | 0.885* | 0.880 | 0.875* | 0.891* |
| **Albumin** | 0.801* | 0.807* | 0.852* | 0.851* | 0.782 ns |
| **Sucrose synthase (SuSy)** | 0.919** | 0.912** | 0.915** | 0.925** | 0.872* |
| **Soluble starch synthase (SSS)** | 0.913** | 0.912** | 0.887* | 0.915** | 0.818* |
| **Acid invertases (AIs)** | 0.872** | 0.851** | 0.805* | 0.882* | 0.751ns |

Correlations among yield parameters, leaf traits, seed storage proteins, seed composition, and enzymes in various chickpea genotypes under combined heat and drought stress. Notably, a significant negative correlation occurred between electrolyte leakage and yield traits, with sensitive genotypes exhibiting higher electrolyte leakage than tolerant genotypes under the combined stress. Other leaf-based characteristics like RLWC, photosynthetic efficiency, and stomatal conductance positively correlated with yield traits. Yield parameters positively correlated with seed storage proteins, seed composition, and enzymes except for a few traits that were not significantly correlated.

* $P < 0.05$;.

** $P < 0.01$; ns: non-significant $P \geq 0.05$.

DT genotype's seeds showed significantly greater activity of soluble starch synthase compared to the DS genotype.

Sucrose synthase activity decreased by 19–48 % under drought stress, 16–17 % under heat stress, and 32–73 % under combined stress compared to the control (Fig. 7c). Seeds of the DT genotype had markedly higher SSy activity than the DS genotype across all stress treatments.

Acid invertase activity decreased by 22–36 % under drought stress, 11–15 % under heat stress, and 41–63 % under the combined stress compared to the control (Fig. 7a). Seeds of the DT genotype had higher acid invertase activity than the DS genotype across all stress treatments.

### 3.4. PCA between leaf and yield traits

PCA of chickpea genotypes under a combination of heat and drought stress revealed correlations between yield parameters [pod number per plant, single seed weight (SSW), total seed weight (TSW), seed-filling rate (SFR), and seed-filling duration (SFD)] and leaf traits [RLWC, EL %, chlorophyll concentration, photosynthetic efficiency (PSII), and stomatal conductance], with PC1 accounting for 93.4 % of the overall variation and PC2 contributing 4.5 %. In PC1, major positive contributors were pod number per plant (0.89), SSW (0.88), RLWC (0.86), and PSII (0.85), whereas EL% had a significant negative impact (−0.91) on yield and physiological traits (Fig. 8).

### 3.5. PCA between seed composition and yield traits

PCA showed that PC1 explained 95.6 % of the total variation, and PC2 contributed 3.5 %. Strong positive correlations occurred between yield traits (pod number per plant, SSW, TSW, SFR, SFD) and seed composition traits (starch, proteins, and soluble sugars), seed storage proteins (albumins, globulins, glutelins, and prolamins), seed carbohydrates (sucrose, glucose, and fructose), and enzymes (acid invertase, sucrose synthase, and soluble starch synthase). Major positive contributors impacting PC1 were fructose (0.87), glucose (0.84), sucrose (0.88), sucrose synthase (0.86), proteins (0.86), glutelins (0.86), starch (0.88), globulins (0.85), pod number per plant (0.91), and SSW (0.91) (Fig. 9).

## 4. Discussion

This study underscores the adverse impact of drought and heat stress during seed filling on various seed yield components, particularly seed quality, in chickpea genotypes. The combined stress exhibited more

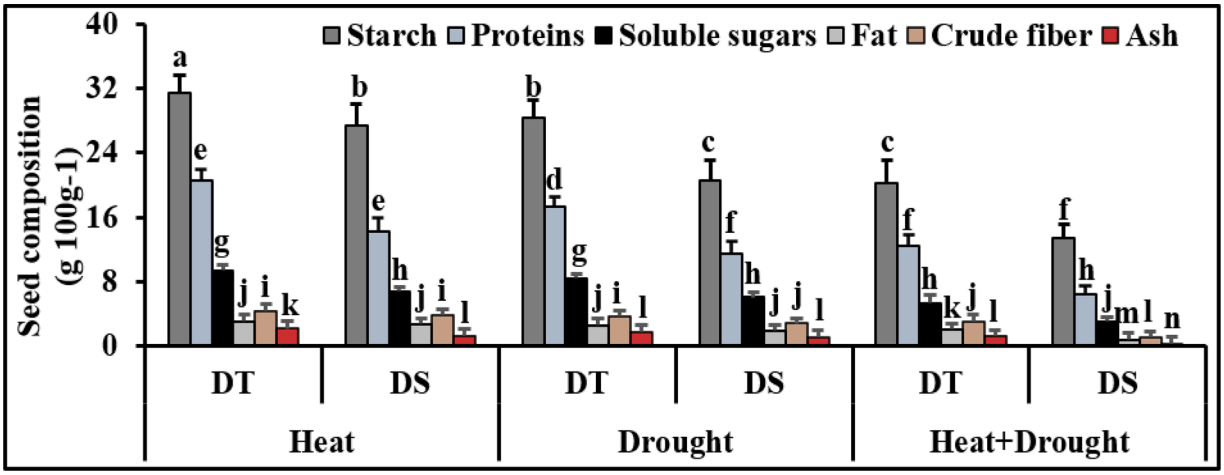


**Fig. 4.** Seed composition (starch, proteins, soluble sugars, fat, crude fiber, and ash) in drought-tolerant (DT) and drought-sensitive (DS) chickpea genotypes exposed to heat, drought, and combined heat and drought stress. LSD ($P < 0.05$): 2.56 (starch), 3.23 (proteins), soluble sugars (2.12), fat (1.26), crude fiber (1.13), ash (1.24). Different letters on bars indicate significant ($P < 0.05$) differences from each other. Different letters on bars indicate significant ($P < 0.05$) differences from each other. Values are mean ± *S*.E. ($n = 3$).

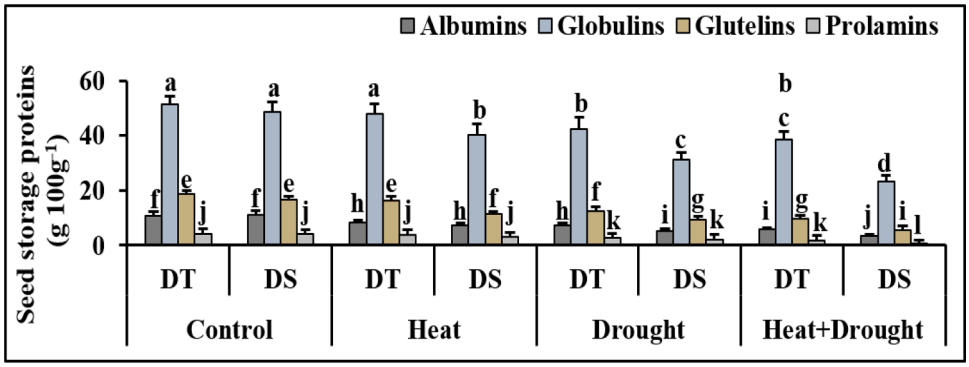


**Fig. 5.** Seed storage proteins (albumins, globulins, glutelins, prolamins) in drought-tolerant (DT) and drought-sensitive (DS) chickpea genotypes exposed to heat, drought, and combined heat and drought stress. LSD ($P < 0.05$): 1.46 (albumins), 6.61 (globulins), 3.15 (glutelins), 0.36 (prolamins). Different letters on bars indicate significant ($P < 0.05$) differences from each other. Values are mean ± *S*.E. ($n = 3$).

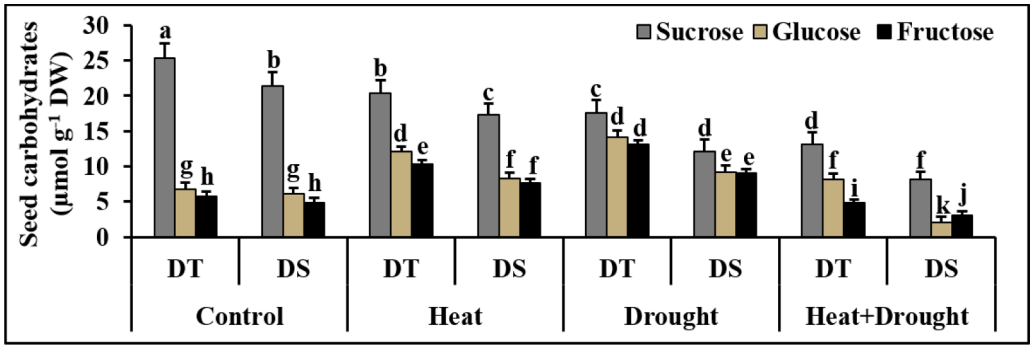


**Fig. 6.** Seed sugars (sucrose, glucose, and fructose) in drought-tolerant (DT) and drought-sensitive (DS) chickpea genotypes exposed to heat, drought, and combined heat and drought stress. LSD ($P < 0.05$): 2.8 (sucrose), 0.65 (glucose), 0.64 (fructose). Different letters on bars indicate significant ($P < 0.05$) differences from each other. Values are mean ± *S*.E. ($n = 3$).

detrimental effects than the individual stresses, substantially reducing the nutritional value of seeds.

### *4.1. Impact on seed number and size*

Seed weight and quantity experienced a notable decrease as a result of pod losses and a reduced number of filled pods, consistent with earlier studies in drought-affected chickpea and lentil ("Behboudian et al., 2001; Sehgal et al., 2017"), as well as in heat-affected chickpea and lentil ("Kaushal et al., 2013; Sita et al., 2017"). The stresses in combination exerted a more pronounced effect on both seed weight and quantity compared to the single stresses, resulting in substantial yield reductions attributed to decreases in biomass, number of pods and seeds, and size of seeds. These results align with studies conducted in wheat and chickpea ("Shah and Paulsen, 2003; Awasthi et al., 2014"), underscoring the significant influence of concurrent heat and drought stress on the size and weight of seeds that was closely related to diminished "photosynthesis" and decreased "water use efficiency." The decrease in size of the seeds noted in this investigation was related to a decline in both the duration and rate of seed filling. Prior studies indicate that the reduction in grain weight under high temperature or water stress throughout the initial stages of grain filling is mainly ascribed to a reduced number of endosperm cells (Nicolas et al., 1985). In contrast, in the later stages of grain filling, disruptions in starch synthesis arise either from limited assimilate availability for growing seeds (Blum, 1998) or from the direct inhibitory influence of these stress treatments on the production of reserve substances (Yang et al., 2004).

### *4.2. Physiological mechanims of seed filling impairment*

The decline in pod and seed numbers and seed weight may be linked to reduced sucrose supply from leaves to developing pods and seeds, as noted in previous studies on chickpea (Awasthi et al., 2014) and lentil (Sehgal et al., 2017). The current study indicates a marked inhibition of seed sucrose content in stressed chickpea plants, particularly under combined stress, potentially impairing the "seed filling rate" and reducing the "seed filling duration," ultimately decreasing the size of the seeds. Similar observations have been reported in barley (Savin and Nicolas, 1996) and wheat (Altenbach, 2012) in the presence of heat and drought stress combinations. Photoassimilation processes in leaves regulate seed filling by delivering sucrose and precursors for starch, protein, and fat synthesis. Therefore, any environmental stress that influences the synthesis and transfer of these molecules from leaf and their consumption in seed would also impact the seed filling processes ("Kaushal et al., 2013; Awasthi et al., 2014"). Leaf water content decreased significantly in the presence of both stresses, potentially impacting cellular functions, consistent with findings in other plant species, such as chickpea (Awasthi et al., 2014), barley (Templer et al., 2017), and a few grasses (Jiang and Huang, 2001), subjected to combined stressors. Moreover, the severe membrane damage to leaf cells, reduced chlorophyll content, and inhibited photosynthetic efficiency under the combined stress could be attributed to the substantial decrease in stomatal conductance and water status in leaves (Awasthi et al., 2014). Membrane damage can occur directly as a result of these stressors ("Guadagno et al., 2017; Horváth et al., 2012") or from oxidative damage under combined stress (Johnson et al., 2014), as noticed in chickpea (Awasthi et al., 2017).

The decline in chlorophyll can be ascribed to leaf water loss,

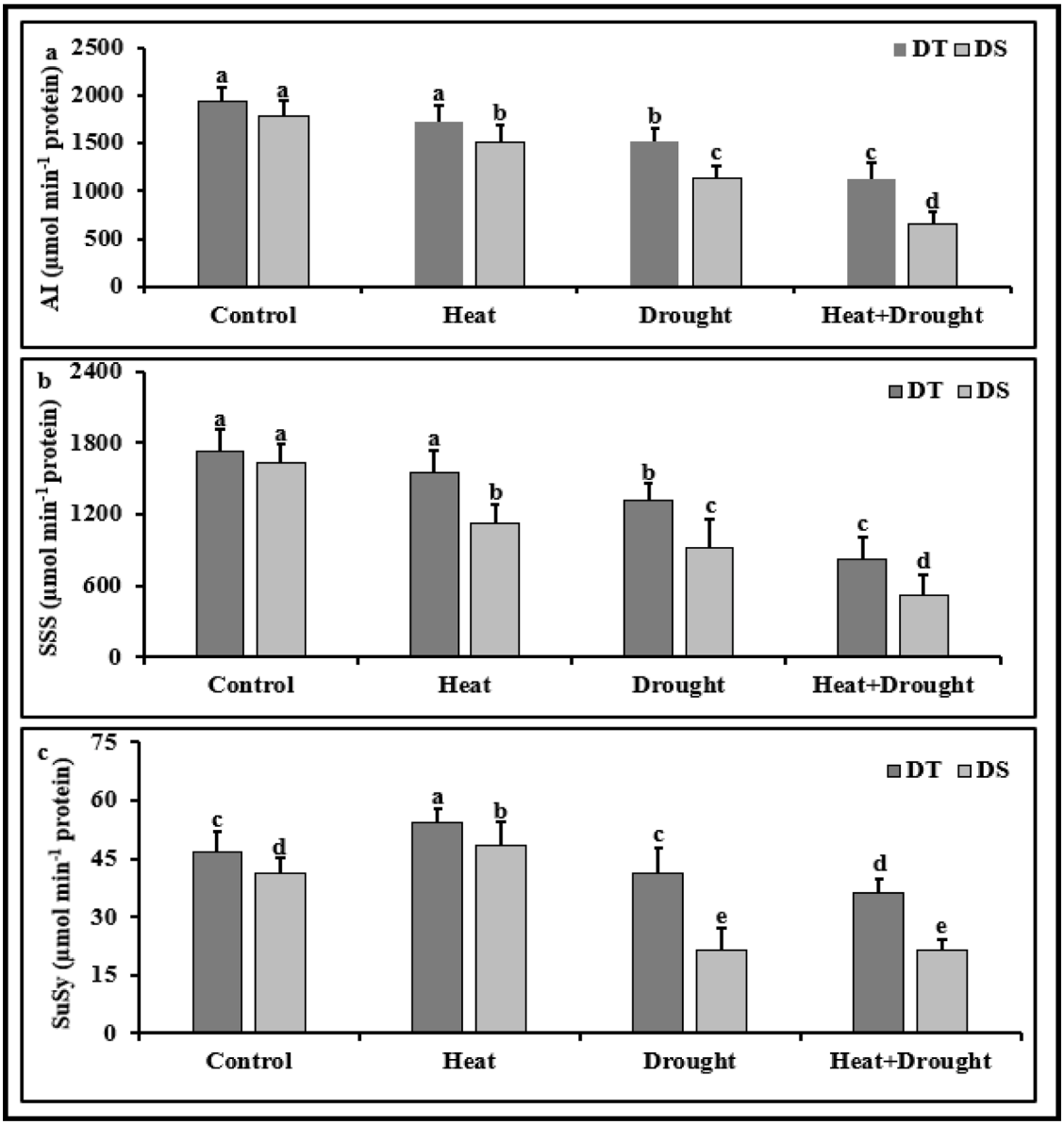


**Fig. 7.** Acid invertase (AI)(a), soluble starch synthase (SSS)(b), and sucrose synthase (SuSy) (c) in drought-tolerant (DT) and drought-sensitive (DS) chickpea genotypes exposed to heat, drought, and combined heat and drought stress. LSD ($P < 0.05$): 258.3 (AI), 114.3 (SSS), and 9.6(SuSy). Different letters on bars indicate significant ($P < 0.05$) differences from each other. Values are mean ± *S*.E. ($n = 3$).

increased photooxidation (Guo et al., 2006), suppressed synthesis, or increased breakdown (Tewari and Tripathy, 1998). These factors correspond to findings in rice, chickpea, and tomato (Kumar et al., 2014; Awasthi et al., 2014; Nankishore and Farrell, 2016) and when subjected to drought and/or heat stress. In this study, chlorophyll fluorescence, serving as a marker of electron transport efficiency during the "light reaction of photosynthesis," decreased due to the impairment of chlorophyll and potentially the proteins in reaction centers. This damage may impair the flow of electrons in the foliage of stressed plants, similar to reports in tomato, Kentucky bluegrass, and chickpea (Nankishore and Farrell, 2016; Jiang and Huang, 2000; Awasthi et al., 2014). The decline in "stomatal conductance, leaf water content," photosynthetic efficiency, and chlorophyll likely reduced photosynthesis, especially under the stress combination, as reported in barley, chickpea, and tomato (Jedmowski et al., 2015; Awasthi et al., 2014; Nankishore and Farrell, 2016).

Under heat stress, stomatal conductance generally rises as an adaptive mechanism, enabling the transpirational cooling of leaves. On the contrary, a reduction in stomatal conductance" in drought stressed plants could be attributed to a decline in leaf water potential (Ludlow and Muchow, 1990) or atmospheric humidity levels (Maroco et al., 1997). The noteworthy reduction in "stomatal conductance" observed in chickpea under the combined stress might be a result of extensive damage to hydraulic conductance and the water status of the leaves. This is evident from the low RLWC values, corresponding to the findings of a previous investigation (Awasthi et al., 2014) in chickpea.

### 4.3. Sucrose and starch synthesis: impacts on yield and seed quality

The enzymes involved in sucrose production in the leaves were impeded by both individual and joint stresses, consistent with prior findings (Awasthi et al., 2014). These stressors may also impede sucrose transporters, disrupting the flow of sucrose to growing seeds (Jain et al., 2007). The insufficient photosynthetic activity to fulfil the assimilate supplies of growing seeds results in a slowed seed growth rate (Sinclair and Rufty, 2012; Ullah et al., 2019). Under drought and/or heat stress,

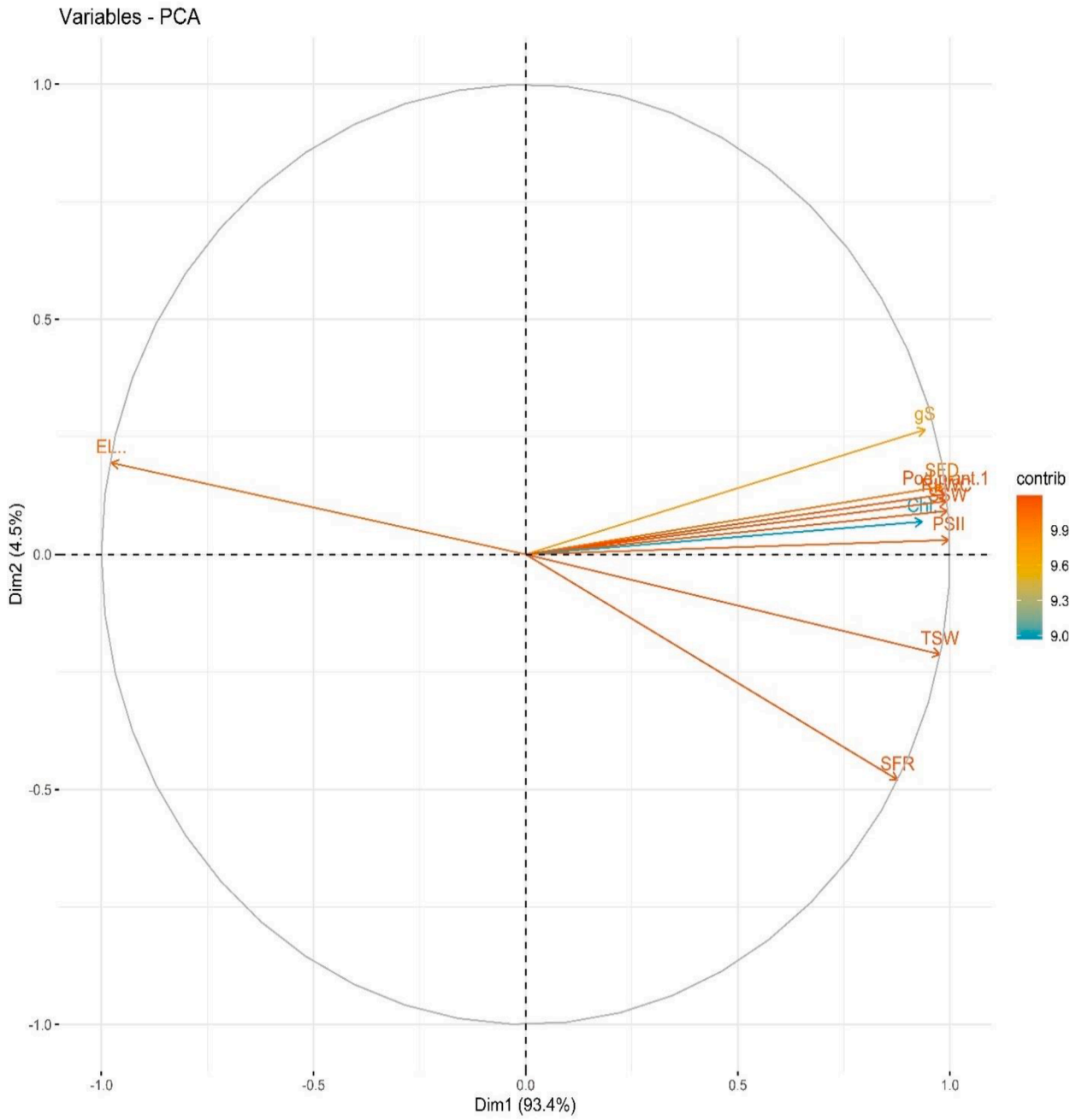


**Fig. 8. Principal component analysis (PCA)** of various traits (leaf and yield) in chickpea genotypes under combined heat and drought stress.
Abbreviations: EL: electrolyte leakage; gS:stomatalconductance; RLWC: relative leaf water content;Chl:chlorophyll content;PSII: photosynthetic efficiency;pod.1: pod plant$^{-1}$; SSW: single seed weight; TSW:total seed weight;SFR: seed filling rate;SFD:seed filling duration.

the reduction in plant growth and the transfer of carbon from leaves to growing seeds exceed the decrease in photosynthesis, further hindering sucrose synthesis in the leaves (Prasad et al., 2017). The reduced availability of sucrose to the growing seeds due to impaired synthesis and transport can have significant consequences for seed yield and quality. Sucrose serves as a primary carbon source for seed filling, and its insufficient supply can lead to decreased seed weight and size (Borrás et al., 2004). This is consistent with the findings of reduced seed weight and quantity observed in this study under combined drought and heat stress.

Starch and sugars are crucial components of seeds. Sucrose can be partially produced in seeds and hydrolyzed into “simple sugars (glucose and fructose)," which are utilised in the starch production process through several enzymes in seeds (Weber et al., 1997). In the present study, heat and drought stress significantly decreased the activity of enzymes related to starch and sucrose synthesis in the seeds, particularly under the combined stress, further reducing the starch and sucrose contents. Studies have revealed that the metabolism of sucrose and starch-related enzymes in seeds is susceptible to heat and drought stress, as observed in sorghum (Bing et al., 2014), maize (Wilhelm et al., 1999), and wheat (“Liu et al., 2011”). Sucrose consumption was likewise disrupted in chickpea seeds, as evidenced by a significant decrease in the activity of “acid invertase” (a sucrose-hydrolyzing enzyme) under concurrent stress, potentially limiting the transfer of sucrose from leaves into growing seeds (Weber et al., 1997). Acid invertases are primary targets of drought stress during maize seed development (Andersen et al., 2002). The reduction in starch and sucrose synthesis not only affects seed weight, but also influences seed quality. Starch serves as a

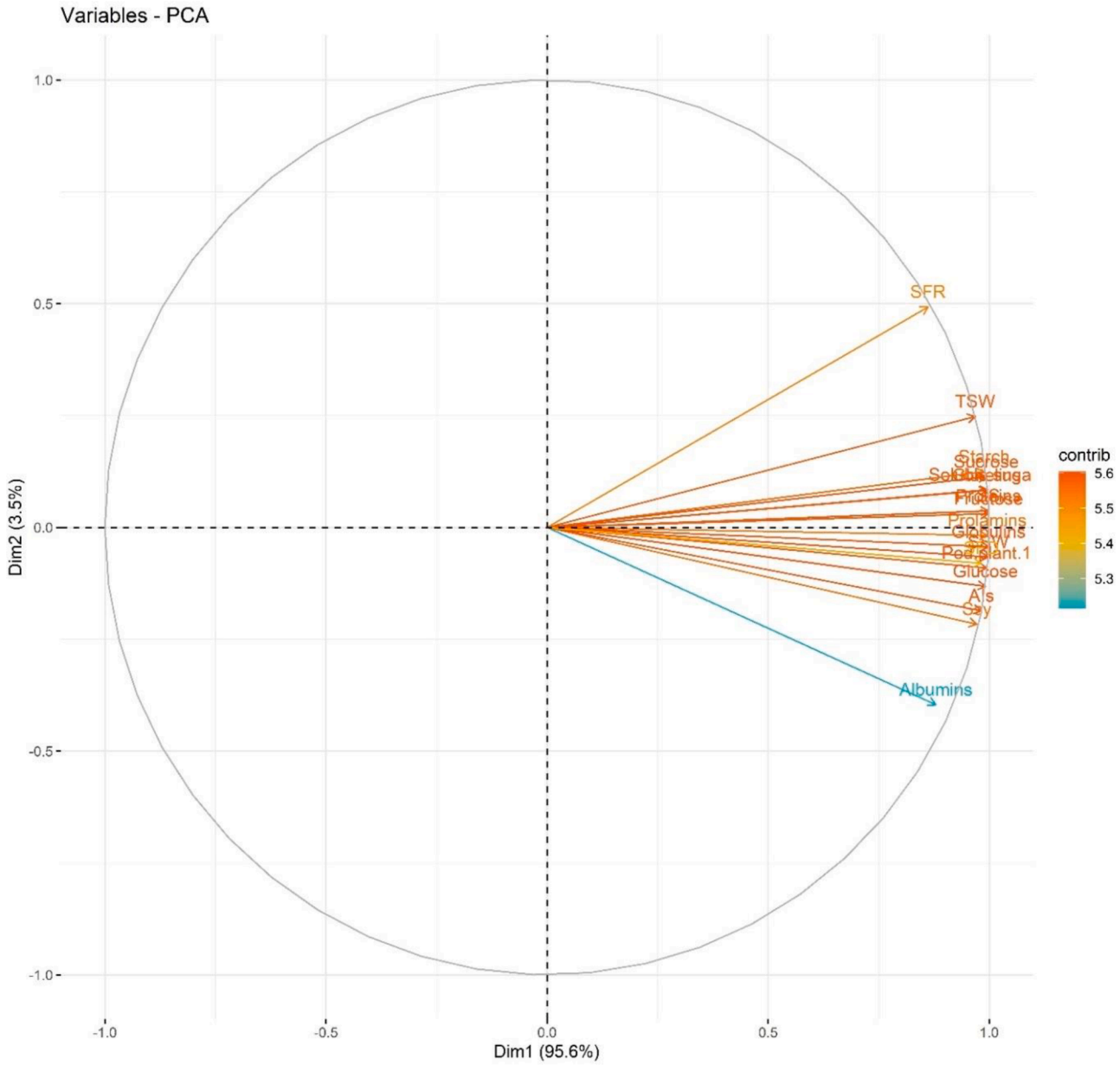


**Fig. 9. Principal component analysis (PCA)** of various traits (seed composition and yield) in chickpea genotypes undercombined heat and drought stress.
**Abbreviations:** SSW: single seed weight; TSW:total seed weight;SFR: seed filling rate;SFD: seed filling duration; SSS: soluble starch synthase;SSY: sucrose synthase; AI: acid invertase.

major energy reserve for germination and early seedling growth, while sugars play essential roles in cellular metabolism and osmoregulation. The observed decline in enzyme activity related to starch and sucrose metabolism under combined stress conditions may lead to lower seed viability and vigor, ultimately affecting crop establishment and yield potential. The mechanisms underlying the inhibition of starch and sucrose synthesis enzymes during stress conditions may involve several factors. Heat stress can lead to protein denaturation and altered enzyme kinetics, while drought stress may result in osmotic stress and reduced availability of substrates necessary for enzyme activity (Wang et al., 2016). Additionally, the accumulation of reactive oxygen species (ROS) during stress can damage cellular components, including enzymes, further impairing metabolic processes (Apel and Hirt, 2004).

Our study highlights that heat and drought stress inhibited the enzyme responsible for synthesising starch (soluble starch synthase) more than the enzyme responsible for synthesising sucrose (sucrose synthase). This observation indicates a disturbance in diverting hexoses towards starch formation, consistent with previous findings in wheat (Ahmadi and Baker, 2001). In drought-stressed wheat plants, the cessation of grain growth was linked to the disruption in activity of enzymes of starch synthesis (Ahmadi and Baker, 2001). The decrease in activity of "sucrose synthase" observed in our study aligns with studies on beans and chickpeas exposed to drought stress (Castrillo, 1992; Behboudian et al., 2001). In chickpea, the activity of sucrose synthase is associated with the size of the seeds (Ashok and Turner, 2009). The levels of "soluble sugars" increased in response to individual heat or drought stress, with glucose and fructose being the primary contributors, likely due to the increased hydrolysis of starch and sucrose. These "soluble sugars" can assist in many functions within cells, including acting as a source of energy, signalling molecules, and osmoprotection (Rosa et al., 2009). Conversely, when plants were exposed to the combined stress, the levels of soluble sugars decreased significantly due to the increased severity of the stress. This reduction may have led to the overall inhibition (Carmo-Silva et al., 2012) of cellular metabolism,

encompassing the synthesis and utilisation of sugars. Furthermore, in the presence of combined stress, the transportation of sucrose from leaves to seeds may be disrupted as a result of the downregulation of sucrose transporters of the tissues of these organs (Qin et al., 2008) to reduce the concentration of "soluble sugars.". The differential inhibition of starch and sucrose synthesis enzymes under stress conditions has important implications for seed quality and yield. The reduced starch synthesis can lead to lower energy reserves in the seeds, potentially affecting germination and seedling vigour (Reed et al. 2022). Additionally, the decreased sucrose synthase activity and disrupted sucrose transport can limit the availability of carbon skeletons for seed filling, resulting in smaller seed size and lower seed weight (Awasthi et al.2014).

### 4.4. Implications for seed quality and nutrition

The combined stress decreased seed storage proteins, particularly albumin and globulins, indicating impaired protein synthesis. This reduction in protein content may result from the inactivation of protein biosynthetic pathways and/or a lack of sufficient precursors. These findings align with previous research on drought-stressed seeds in bean, chickpea and wheat (Ghanbari et al., 2015; Behboudian et al., 2001; Begcy and Walia, 2015). The reduction in seed storage proteins can have significant implications for seed quality and nutritional value as seed storage proteins are a major source of dietary protein for humans and livestock. The observed decrease in albumin and globulin fractions may reduce the overall protein content and alter the amino acid composition of chickpea seeds, potentially affecting their nutritional quality. Further research is needed to elucidate the specific mechanisms underlying the inhibition of protein synthesis under combined stress conditions. Investigating the expression and regulation of genes encoding enzymes involved in protein biosynthesis can provide valuable insights.

### 4.5. Response of contrasting genotypes

Heat and/or drought stress resulted in less damage to seed yield and quality in the DT genotypes than the DS genotypes possibly due to increased leaf stability under stress conditions, leading to a reduction in damage to "photosynthetic activity" and "cell membranes". The DT genotypes retained more water in its leaves, contributing to better performance and facilitating increased sucrose synthesis and transport into developing seeds. Consequently, the DT genotypes had higher seed filling rates, longer filling durations, and larger seed sizes than the DS genotypes, resulting in a more modest impact on the accumulation of seed reserves. Moreover, the DT genotype exhibited superior performance across all stress treatments, indicating cross-tolerance. Previous studies in crops like chickpea (Awasthi et al., 2014) and lentil (Sehgal et al., 2017) have similarly highlighted the advantage of DT genotypes in maintaining higher leaf water status under stress conditions. Further investigations into turgor maintenance, sucrose transport, and seed metabolism mechanisms are needed to explore why the DT genotypes exhibits greater stability than the DS genotype. The identification of DT genotypes with superior performance under combined heat and drought stress conditions has important implications for breeding programmes and crop management strategies.

PCA conducted on chickpea genotypes under a combination of heat and drought stress conditions offered valuable insights into the relationships between yield parameters and leaf traits. PC1 emerged as the primary component, explaining a significant portion of the overall variation, while PC2 made a comparatively lesser contribution. Positive contributors to PC1, including pod number per plant, single-seed weight, RLWC, and PSII, underscored the pivotal role of these variables in enhancing yield and physiological traits. In contrast, electrolyte leakage negatively affected yield and physiological parameters. These findings highlight the significance of managing membrane integrity and maintaining favourable leaf water content for the optimal performance of chickpea genotypes exposed to combined heat and drought stress. PCA biplot can serve as a valuable tool for identifying superior chickpea genotypes based on their performance under combined heat and drought stress. Genotypes that cluster closer to the positive end of PC1, characterised by high pod number, single-seed weight, RLWC, and PSII, can be considered as potential candidates for further evaluation and selection. PCA of yield traits and seed composition highlighted the significant contributions of fructose, glucose, sucrose, sucrose synthase, proteins, glutelins, starch, globulins, pod number per plant, and single-seed weight to PC1. These findings indicate that the presence of soluble sugars, proteins, and starch, coupled with the activities of associated enzymes, positively affects yield traits and seed quality in chickpea genotypes.

## 5. Conclusion and potential avenues

The observations of the present study revealed that combined heat and drought stress have a synergistic negative impact on chickpea, affecting various mechanisms such as disruption of water relations, photosynthesis, inhibition of key enzymatic processes, and transport of precursors related to seed filling. Consequently, it resulted in drastic reductions in seed yield and nutritional quality compared to individual stresses. This study also revealed that the DT genotype exhibits greater resilience than the DS genotype due to its enhanced leaf stability, water retention, superior sucrose synthesis and transport capabilities. PCA indicated the critical importance of maintaining leaf water status and membrane integrity for ensuring good yield performance in chickpea genotypes exposed to combined heat and drought stress. It also highlighted the critical roles of soluble sugars, proteins, starch, and their associated enzymes in influencing yield traits and seed quality, emphasising the importance of maintaining optimal levels of these components for improved performance under combined stress conditions. The identification of key traits and their relationships can assist breeding efforts and agronomic practices aimed at enhancing chickpea seed characteristics and yield potential. Breeding programmes can benefit from focusing on genotypes with higher seed composition parameters and stress tolerance traits. Furthermore, future research should explore the genetic and physiological mechanisms underlying these relationships to develop effective strategies for enhancing chickpea resilience and productivity in the face of climate change. This information can also be used to develop agronomic practices such as mulching or irrigation management as well as application of growth regulators to alleviate stress effects, which could also be beneficial for improving chickpea yield in stressed environments. Moreover, the optimisation of nutrient management to enhance seed composition and yield in chickpea in stressed environments may also be explored.

## CRediT authorship contribution statement

**Rashmi Awasthi:** Writing – original draft, Methodology, Investigation, Formal analysis. **Poonam Devi:** Writing – original draft, Methodology, Investigation, Formal analysis. **Uday Chand Jha:** Writing – review & editing, Writing – review & editing. **Kamal Dev Sharma:** Writing – review & editing. **Manish Roorkiwal:** Writing – review & editing. **Sanjeev Kumar:** Writing – review & editing. **Ashwani Pareek:** Writing – review & editing. **Kadambot H.M. Siddique:** Writing – review & editing. **PV Vara Prasad:** . **Swarup K. Parida:** Writing – review & editing. **Harsh Nayyar:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100635.

## Data availability

Data will be made available on request.

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