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RESEARCH ARTICLE

Drought-induced regulation of chalcone synthase activity in cress (*Lepidium sativum* L.) leaves

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Abstract

Drought is a major abiotic stressor that severely limits plant growth and development. In response, plants often enhance the biosynthesis of flavonoids, key secondary metabolites that contribute to growth regulation and stress adaptation. This study investigated the drought tolerance mechanisms of cress (*Lepidium sativum* L.) by examining the effects of varying drought durations on growth parameters, stress severity and associated metabolic responses. The results showed that drought stress significantly impaired plant growth, with shoot and root lengths reduced to 0.7- and 0.8-fold of control levels, respectively, after 4 days of water deficit. Prolonged drought (12 days) further decreased relative water content (RWC), reaching 0.8-fold in shoots and 0.6-fold in roots. Stress severity, assessed by relative electrolyte conductivity, exhibited a nine-fold increase after 12 days, indicating considerable membrane damage. Biochemical analyses revealed a time-dependent increase in total flavonoid content, which rose by 1.3-, 1.6- and 1.4-fold after 4, 8 and 12 days of drought exposure, respectively. Anthocyanin content increased modestly by 1.6-fold after 12 days of drought. Chalcone synthase (CHS) activity increased markedly, by 1.9- and 2.7-fold after 4 and 8 days, respectively, indicating its early induction as a critical drought response. Overall, these findings underscore the importance of CHS activation and flavonoid accumulation in enhancing drought resilience in cress. Further studies should focus on identifying individual flavonoid compounds and clarifying their role in maintaining membrane integrity under prolonged drought conditions.

Keywords: chalcone synthase; drought; stress severity; tolerance

Introduction

Lepidium sativum L., commonly known as garden cress and a member of the Brassicaceae family, is highly adaptable to various soil types and climatic conditions, facilitating its widespread cultivation (1). Throughout their lifecycle, plants frequently face abiotic stresses like drought and salinity, which disrupt cellular homeostasis by causing osmotic and turgor imbalances. These stresses alter ion distribution, gene expression and metabolic processes, ultimately affecting growth and productivity (2-11). Specifically, drought reduces leaf water potential and turgor pressure, impairs photosynthesis, chlorophyll synthesis, nutrient metabolism and carbohydrate production and triggers stomatal closure, all of which inhibit plant development (6-8).

Flavonoids are key secondary metabolites involved in plant growth, defense and responses to environmental stress. Their biosynthesis is regulated by chalcone synthase (CHS, EC 2.3.1.74), the primary enzyme initiating the flavonoid pathway. CHS activity plays a dual role in plant adaptation to stresses such as drought, UV radiation, wounding and pathogen attack (12-14). While CHS regulation under abiotic stress has been studied in various plants and organs, its specific role and regulation in *L. sativum* remain unclear. Moreover, plant stress responses also modulate levels of signaling molecules like salicylic acid, which affect flavonoid production and overall stress tolerance (15).

Stress conditions generally reduce photosynthetic pigment synthesis, decreasing light capture and energy production, which are closely linked to biomass yield (16). Plants adapt to stress through a combination of morphological, physiological and biochemical mechanisms; however, some fail to manage these challenges, resulting in impaired water transport and reduced pigment production, ultimately leading to plant decline (17). Under drought, plants activate osmoregulatory pathways that accumulate organic solutes like sugars, amino acids and hydrophilic proteins to maintain membrane integrity and water balance (18, 19).

This study aims to investigate the effects of drought stress on flavonoid biosynthesis in *L. sativum* by focusing on CHS activity, flavonoid content and salicylic acid levels during varying durations of water-deficit exposure. We hypothesize that drought stress modulates CHS regulation in garden cress, leading to altered flavonoid accumulation as part of its adaptive response.

Materials and Methods

Plant materials and growth conditions

Cress (*L. sativum*) plants were grown under controlled conditions (14 hr light with 54 µE and 21 °C/10 hr dark and 20 °C, 55-60 % relative humidity) in 2:1:1 peat moss, perlite and vermiculite,

respectively. Trays filled with cultured pots were transferred to a growth chamber under controlled conditions. Six weeks after germination, some plants were exposed to drought with different exposure times; the other plants were kept in growth chamber conditions without any changes. Each treatment was conducted in triplicate, comprising a total of 90 samples and leaves from individual plants within each replicate were pooled to provide representative samples for subsequent physiological and biochemical analyses.

Drought stress treatments

Plants were grown under controlled conditions and irrigated with tap water three times per week for 6 weeks. Thereafter, 6-week-old plants were subjected to drought stress by withholding water for 4, 8 or 12 days. At the end of each treatment, leaf samples were collected, flash-frozen in liquid nitrogen and stored at −80 °C. Age-matched control plants were irrigated throughout the same periods.

Growth parameters

The root and shoot length, fresh weight for root and shoot and relative water content (RWC) in root and shoot of seedlings were measured on 4, 8 and 12 days of drought stress.

Assessment of the relative water content (RWC) of the leaf and root

RWC of the shoot and root was measured using the first fully expanded leaf of four plants per treatment. Cut shoots and roots were immediately weighed to obtain their fresh masses (Fresh Weight, FW) and then shoots and roots were immersed in deionized water in a petri dish and incubated for 48 hr. Shoots and roots were re-weighed to obtain their Turgid Weight (TW). Next, they were dried for 96 hr in an oven at 60 °C, then weighed again to determine the dry weight (DW). Finally, RWC was calculated as (FW − DW)/(TW − DW) × 100 %.

Samples collection and preparation for analysis

The samples (leaves) were transported in liquid nitrogen and stored in a deep freezer (−80 °C). The frozen leaves were ground in liquid nitrogen to be used in analysis.

Determination of total flavonoid content (TFC)

To evaluate TFC, leaf samples (100 mg) were ground and homogenized in 5 mL of 80 % methanol. The samples were incubated on an orbital shaker at a rotation speed of 200 rpm at room temperature for 2 hr and then centrifuged at 8000 × *g* for 5 min. The supernatant was transferred into a new tube and the pellet was extracted one more time. Then, the supernatants were combined for the measurement of TFC (20).

Total flavonoid determination was carried out according to the $AlCl_3$ method (21). A total of 100 µL of crude extract was added to a test tube containing 100 µL of 2 % $AlCl_3$, 20 µL of glacial acetic acid and 200 µL of 100 % methanol. Then, they were mixed well and incubated for 30 min at room temperature. The optical density of each sample was detected at 425 nm. Concentrations of 0.5, 1, 2, 5, 10 and 20 µg/mL of quercetin were used for the calibration curve.

Anthocyanin content

Anthocyanin content was determined by extracting leaf tissue (20 mg) with 1 mL of extraction solvent (methanol:HCL:water (90:1:1)) for 1 hr in the dark at room temperature. Then the homogenized extract was centrifuged at 16240 x *g* for 15 min at 4 °C. The supernatants were transferred into a new tube and the absorbance was measured at 529 nm and 650 nm for the detection of anthocyanin content (22).

Chalcone synthase (CHS) activity assay

CHS enzyme was extracted from 0.4 g of leaves with a solution of 1 mM 2-mercaptoethanol dissolved in 0.1 M borate buffer (1 mL, pH 8.8) at 4 °C. Subsequently, Dowex l × 4 resin (0.1 g) was added to the solution and the mixture rested for 10 min. The solution was then centrifuged at 15000 rpm for 10 min to remove the resin. The supernatant was transferred to a tube and Dowex resin (0.2 g) was added and the mixture was left standing for 20 min. The resin was removed from solution by centrifugation at 15000 rpm for 15 min. The supernatant (100 µL) was mixed gently with 10 mM potassium cyanide and following that, Tris-HCl buffer (1.89 mL, pH 7.8) was added. Subsequently, chalcone (10 mg) was added to ethylene glycol monomethyl ether (10 µL), mixed with the enzyme extract and the reaction was allowed to proceed for 1 min at 30 °C. The absorbance was measured at 370 nm (23).

Estimation and evaluation of salicylic acid (SA)

SA was measured according to Ahmed et al., 2021 (20). Leaf tissue (0.1 g) was homogenized in 1 mL of dH_2O. After centrifugation at 10000*g* for 10 min, the supernatant was stored on ice for SA measurement. A volume of 500 µL of the supernatant was transferred into a new tube with 2.5 mL of freshly prepared 0.1 % ferric chloride. An iron complex is formed after the reaction of ferric acid with aqueous salicylic acid, which gives a violet color. The spectrophotometer was used to measure the absorbance at 540 nm.

Measurement of relative electrolyte conductivity (ELC)

ELC was measured according to Su et al., 2015 (24). Briefly, leaf samples subjected to drought stress, along with their corresponding controls, were placed in 6 mL of distilled water for incubation. The samples were shaken at 0.5 g and 25 °C for 3 hr, after which the initial conductivity (*C*1) was measured using a Multi 9310 EC-pH meter (WTW, Germany). Following this, the samples were autoclaved at 121 °C for 20 min. Once they cooled to room temperature, the conductivity was measured again as *C*2. ELC was then calculated using the formula:

$$ELC\ (\%) = C1/C2 \times 100$$

Statistical analysis

Three separate biological experiments were carried out, with results expressed as mean ± SD from three replicates. Statistical evaluation was done using ANOVA at a 95 % confidence interval ($P \leq 0.05$), followed by Tukey's HSD test to determine significant differences between means. All analyses were performed using Origin software (OriginLab, USA).

To explore relationships between variables, Pearson correlation analysis was carried out between chalcone synthase (CHS) activity and anthocyanin content, as well as between CHS activity and total flavonoid content (TFC). Correlation coefficients (*r*) and corresponding *p*-values were calculated to assess the strength and significance of associations.

Results

Cress plants growth parameters

Fig. 1 illustrates the changes in growth parameters of cress plants in response to drought stress. Drought stress has a significant impact on plant growth, particularly on the length of shoots and roots. Following drought exposure, shoot length was reduced to approximately 0.7-fold the length of the control group (Fig. 1A). Likewise, root length decreased to approximately 0.8-fold that of the control group after 4 days of exposure. Still, this reduction is partially recovered after 8 and 12 days of drought exposure (Fig. 1B). Furthermore, drought stress significantly lowers shoot RWC, reaching approximately 0.8-fold the RWC of the control group after 12 days of drought exposure (Fig. 2A). A similar effect is observed in root RWC, which diminishes to approximately 0.6-fold the RWC of the control group after 12 days of exposure (Fig. 2B). These findings indicate that drought stress adversely affects growth parameters, especially during the early stages of exposure and impacts RWC at longer durations of exposure (Fig. 2).

Leaves relative electrolyte conductivity (ELC)

The assessment of drought tolerance can be achieved by measuring ELC. This method allows for the prompt detection of physiological responses in plants and enables differentiation between various levels of tolerance. Under drought stress conditions, the ELC is influenced, resulting in a slight increase. After 4 and 8 days of exposure, the ELC shows a modest rise, reaching approximately a 2-fold increase compared to the control group. However, after 12 days of exposure, the ELC exhibits a substantial 9 -fold increase compared to the control group, as depicted in Fig. 3.

Leaves total flavonoid content (TFC)

The impact of drought stress on TFC of cress leaves is depicted in Fig. 4. Throughout this study, it was observed that TFC was progressively influenced by drought stress. The severity of drought stress directly correlated with an increase in TFC levels. Specifically, after 4, 8 and 12 days of exposure, the TFC showed significant augmentation of approximately 1.3-, 1.6- and 1.4-fold, respectively, compared to the control condition.

Leaves anthocyanins content

Among the various factors considered in the drought treatment, the highest impact was observed on the content of anthocyanins. Notably, there was a slight but significant increment in the anthocyanins content, reaching approximately a 1.6-fold increase after 12 days of exposure compared to the control condition (Fig. 5).

Leaves salicylic acid (SA) content

Under drought stress, there was no significant response observed in the production of SA and its levels remained unchanged as the duration of stress prolonged. In the cress leaves of all plant treatments, the SA content remained relatively constant at approximately 46-47 ng/g DW (Fig. 6).

Total leaves protein content

The protein content experienced a significant reduction during the drought treatment period. After 4, 8 and 12 days of drought treatment, the protein content decreased by approximately 0.53-, 0.67- and 0.5-fold, respectively, compared to the protein content in the control group (Fig. 7).

Cress plant leaves chalcone synthase (CHS) activity

The impact of drought stress on the activity of CHS in cress leaves was found to be significant in this study. Fig. 8 illustrates that the CHS enzyme activity increased by approximately 1.9- and 2.7-fold after exposure to 4 and 8 days of drought stress, respectively, compared to non-drought stressed plants.

Correlation analysis between CHS activity and flavonoid/ anthocyanin content

Pearson correlation analysis revealed no significant association between CHS activity and anthocyanin levels ($r = 0.09$, $p = 0.74$). In contrast, CHS activity showed a strong positive trend with total flavonoid content ($r = 0.77$, $p = 0.07$), suggesting a potential link between CHS induction and enhanced flavonoid biosynthesis under drought stress.

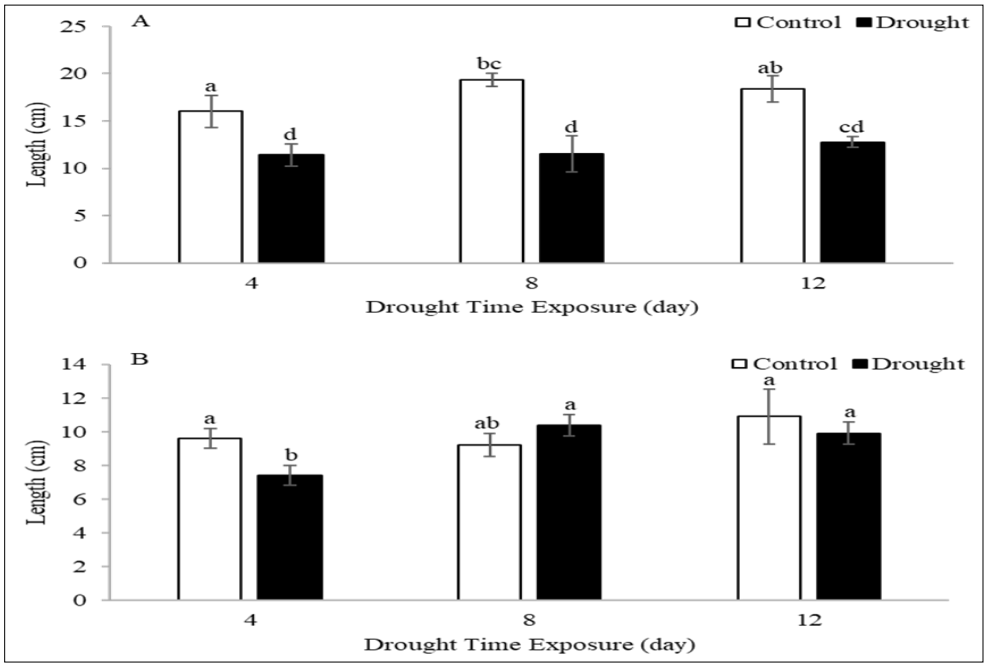

Fig. 1. Shoot (A) and root (B) lengths of cress plants under drought stress at different time points compared with controls. Data represent mean ± SD; $n = 5$. Bars with different letters indicate significant differences (Tukey's test; $P \leq 0.05$).

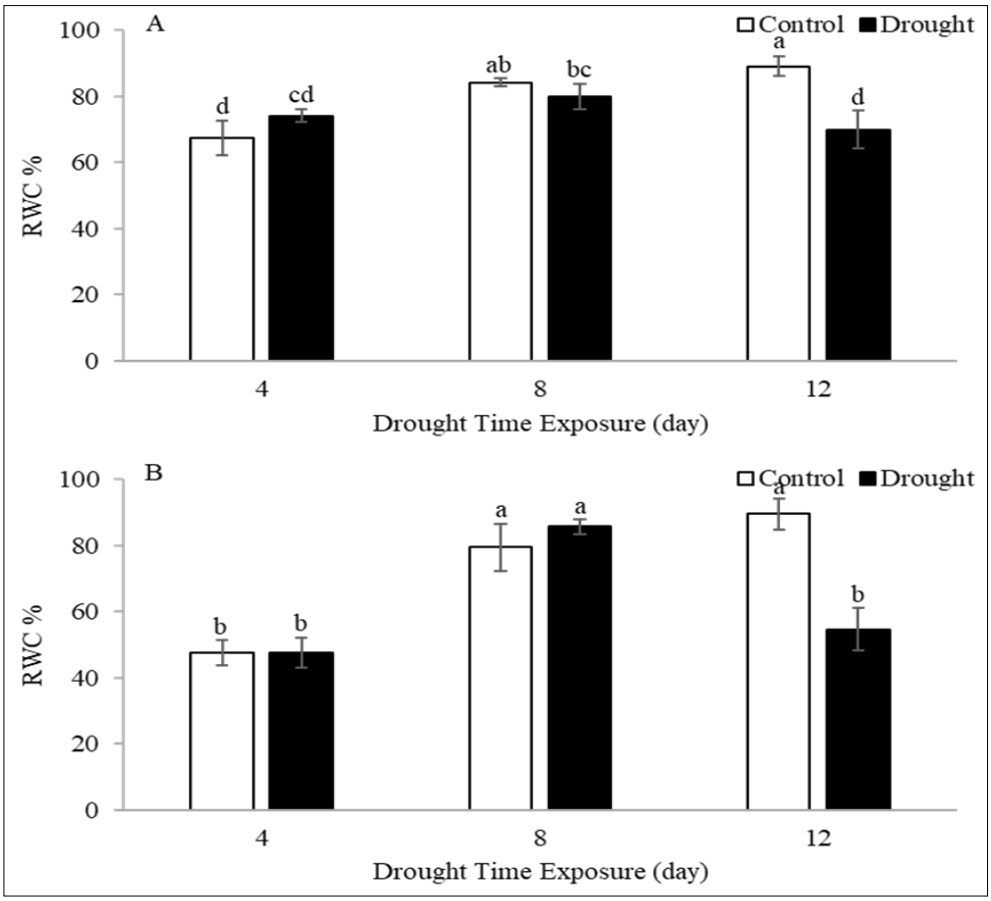

Fig. 2. Relative water content (RWC) of shoots (A) and roots (B) of cress plants under drought stress compared with controls. Data represent mean ± SD; $n = 5$. Bars with different letters indicate significant differences (Tukey's test; $P \leq 0.05$).

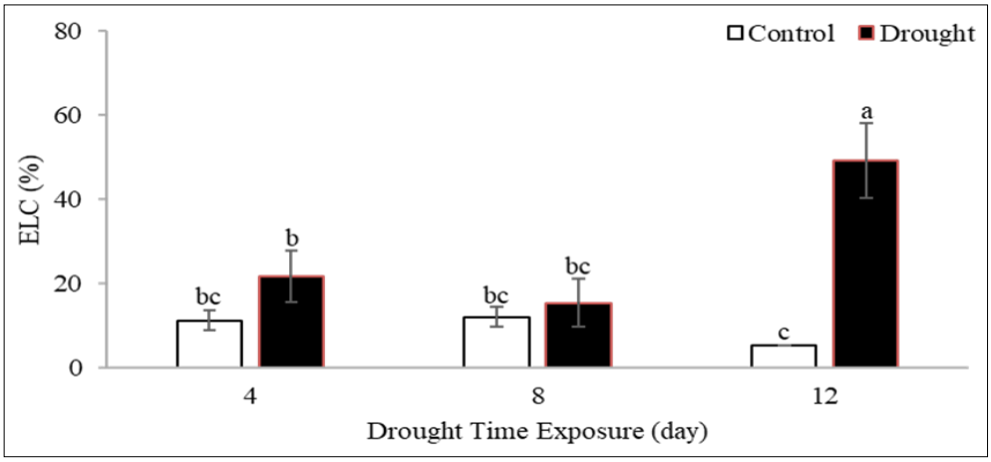

Fig. 3. Relative electrolyte conductivity (ELC) of cress leaves under drought stress compared with controls. Data represent mean ± SD; $n = 4$. Bars with different letters indicate significant differences (Tukey's test; $P \leq 0.05$).

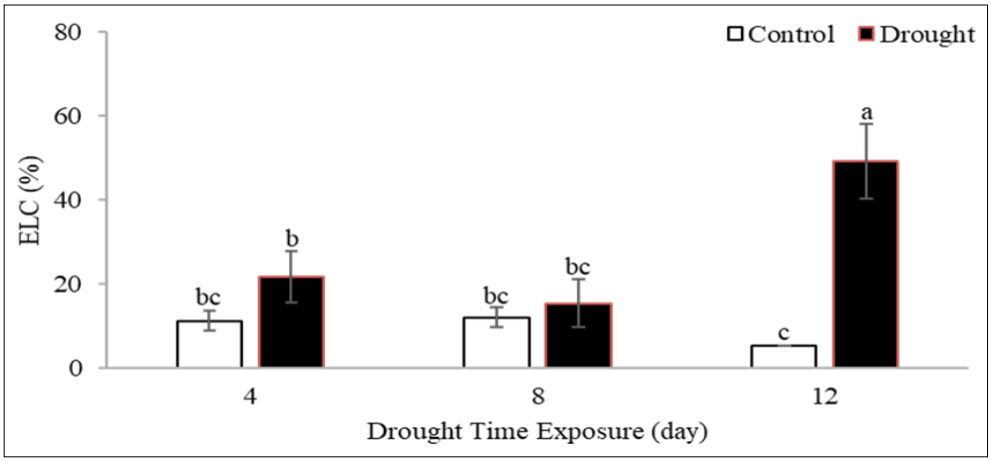

Fig. 4. Total flavonoid content (TFC) of cress leaves under drought stress compared with controls. Data represent mean ± SD; $n = 7$. Bars with different letters indicate significant differences (Tukey's test; $P \leq 0.05$).

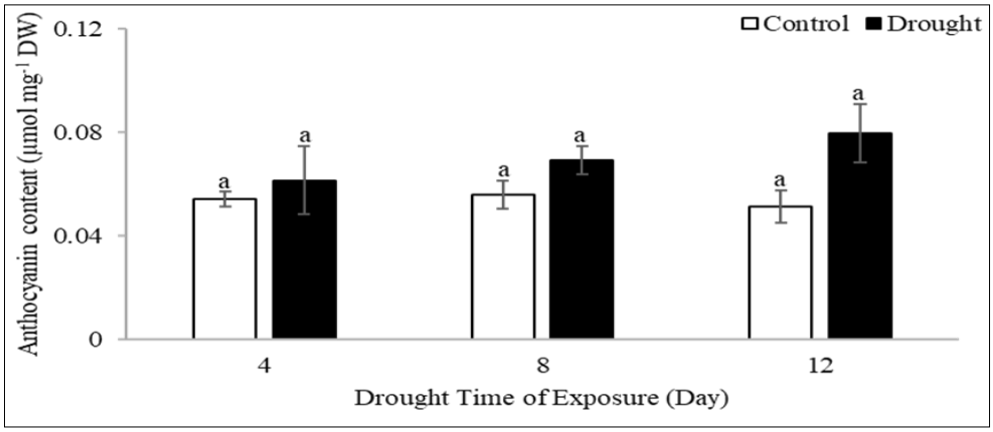

Fig. 5. Anthocyanin content of cress leaves under drought stress compared with controls. Data represent mean ± SD; n = 7. Bars with different letters indicate significant differences (Tukey's test; $P \leq 0.05$).

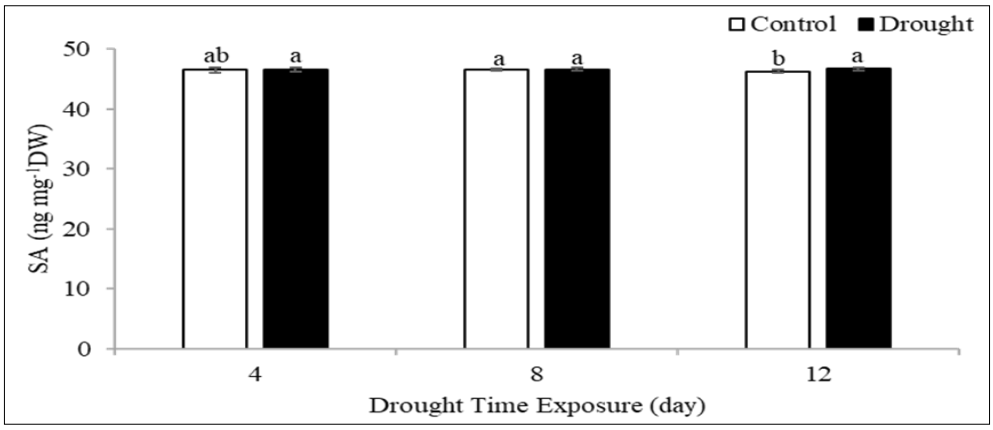

Fig. 6. Salicylic acid (SA) content of cress leaves under drought stress compared with controls. Data represent mean ± SD; n = 7. Bars with different letters indicate significant differences (Tukey's test; $P \leq 0.05$).

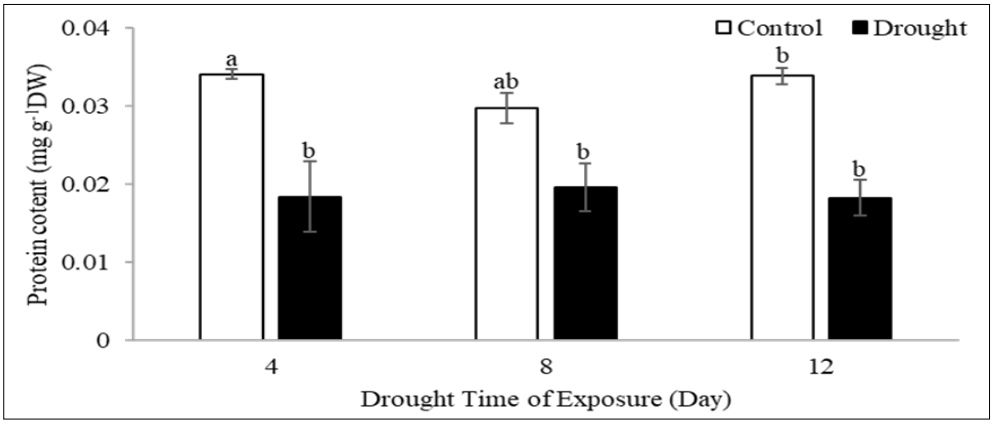

Fig. 7. Protein content of cress leaves under drought stress compared with controls. Data represent mean ± SD; n = 7. Bars with different letters indicate significant differences (Tukey's test; $P \leq 0.05$).

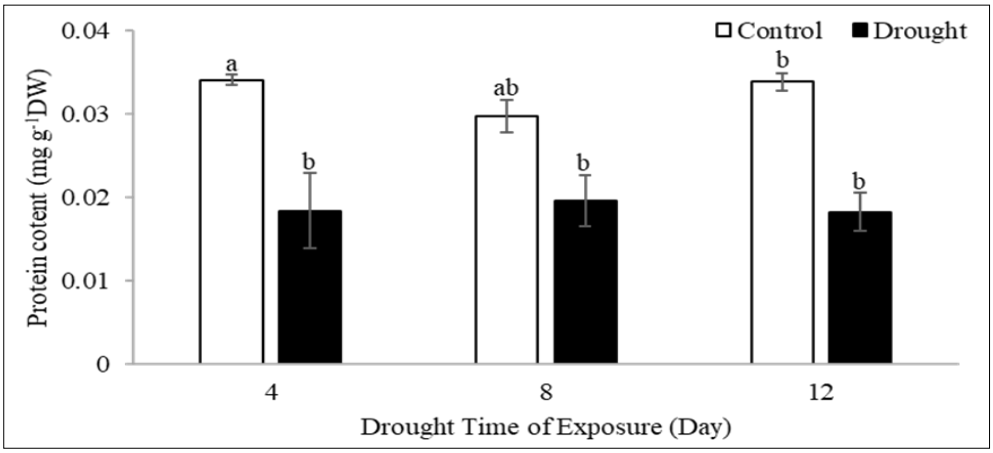

Fig. 8. Chalcone synthase (CHS) enzyme activity in cress leaves under drought stress compared with controls. Data represent mean ± SD; n = 4. Bars with different letters indicate significant differences (Tukey's test; $P \leq 0.05$).

Discussion

Drought is a harsh environmental condition that strongly impacts crop growth and productivity. Water scarcity impairs plant development in ways that depend on stress severity, duration and developmental stage, with effects manifesting through morphological, physiological, biochemical and molecular processes (25). In this study, drought stress reduced shoot and root growth, likely due to limited tissue hydration and reduced turgor pressure, which inhibit cell expansion and division (26-29). Because cell enlargement is particularly sensitive to water deficit (30), reduced shoot and root length may primarily reflect suppressed cell elongation caused by decreased turgor. RWC decreased with prolonged drought exposure (12 days), confirming water deficit at the tissue level (31). Similarly, electrolyte leakage (ELC) increased with stress duration, indicating compromised membrane stability (32). Together, these parameters highlight the negative impact of water limitation on cell expansion, hydration status and membrane integrity.

Flavonoids accumulated progressively under drought conditions, consistent with previous reports that associate flavonoids with stress adaptation mechanisms such as antioxidant activity, osmotic adjustment and regulation of stomatal movement (33-37). Anthocyanin levels also increased after prolonged drought, in agreement with earlier findings that anthocyanins act as protective pigments, mitigating oxidative stress under abiotic stress conditions (38-41). This metabolic shift suggests a reallocation of resources toward stress resilience.

Interestingly, the unchanged SA levels might indicate a SA-independent tolerance mechanism. This contrasts with findings in some other species, where drought elevates SA levels to enhance antioxidative defense and osmotic balance (42, 43). One possible explanation is that *L. sativum* may rely more heavily on flavonoid and anthocyanin accumulation rather than SA signaling to mitigate drought stress. Alternatively, SA regulation might be organ-specific or transient, with changes occurring earlier than the sampling intervals used here. Future time-course studies with finer resolution could clarify whether SA plays a delayed or tissue-specific role in *L. sativum* drought responses.

Total protein content is typically reduced in response to drought stress as an early response (44). CHS is frequently induced under abiotic stress in many plant species, contributing to the accumulation of phenolics, flavonoids, anthocyanins and related compounds (13, 35, 45). Recent studies provide further support for this role: overexpression of a sweet cherry CHS gene (CpCHS1) in tobacco enhanced drought tolerance, confirming the functional importance of CHS in stress adaptation (46). Multi-omics analyses in soybean and *Tetrastigma hemsleyanum* have likewise demonstrated that salt and drought stresses upregulate CHS and other flavonoid biosynthetic genes in leaves, correlating with increased flavonoid accumulation (47, 48). Moreover, CHS regulation is increasingly recognized as complex, with gene family diversification, tissue-specific expression and post-transcriptional regulation by transcription factors and non-coding RNAs shaping its responses to abiotic stress (49, 50). In this study, CHS activity was more strongly stimulated under drought stress than in the controls, suggesting that the increase in CHS activity likely contributed to the observed elevation in total flavonoid content, although causality requires further validation (51). This interpretation is supported by a positive correlation between CHS activity and total flavonoid content (r = 0.77, p = 0.07), whereas CHS activity showed no significant correlation with anthocyanin levels (r = 0.09, p = 0.74).

This study was limited to biochemical measurements at selected drought intervals. Further work should examine transcriptomic changes in flavonoid pathway genes, perform metabolite profiling to identify specific flavonoid subclasses and explore spatial patterns of CHS activity or comparative studies with tolerant and sensitive cultivars. In addition, investigating whether SA dynamics differ between leaves, stems and roots—or at earlier drought stages—would clarify its role in *L. sativum*. Controlled experiments using exogenous SA applications could also help determine whether this pathway contributes to drought resilience in this species.

Conclusion

Cress plants exposed to drought stress exhibited reduced growth parameters and RWC, accompanied by increased electrolyte leakage, flavonoid and anthocyanin accumulation and enhanced CHS enzyme activity. In contrast, salicylic acid levels remained unchanged and total protein content decreased under stress conditions. These findings suggest that *L. sativum* relies primarily on CHS-mediated flavonoid biosynthesis, rather than SA signaling, as a key strategy for drought resilience. Further investigation into phytochemicals, antioxidant enzymes and non-enzymatic factors is required to fully understand how metabolic adjustments contribute to membrane stability under prolonged water deficit. Overall, this study underscores the significance of CHS regulation in enhancing drought tolerance in *L. sativum*. These findings highlight CHS as a potential target for improving drought tolerance in crops.

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Authors' contributions

HA performed the research. KA and TH designed the project, supervised the study, discussed the results and wrote the manuscript. The percentage of participation was equal for all co-authors. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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