

Exogenous proline modulates heat shock and dehydrin protein expression in drought-stressed *Capsicum annuum* L. cv. Yalova Çorbacı 12

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ABSTRACT

Drought stress is a major abiotic constraint limiting crop productivity worldwide, necessitating effective strategies to enhance plant tolerance. This study investigated the effects of exogenous proline application (0, 5, 10, or 20 mM) on physiological responses and stress-associated protein accumulation in pepper (*Capsicum annuum* L.) under drought stress conditions simulated by 10% polyethylene glycol (PEG-6000). Non-stressed or drought-stressed plants were treated with 0, 5, 10, or 20 mM proline. Drought stress significantly increased ion leakage and turgor loss while reducing relative water content (RWC) and total soluble protein (TSP) levels, indicating membrane destabilization and impaired protein homeostasis. Exogenous proline mitigated these effects in a concentration-dependent manner, with 5 and 10 mM treatments most effectively improving membrane stability and plant water status under drought conditions. Endogenous proline accumulation markedly increased under drought stress; however, exogenous application resulted in comparatively lower endogenous levels than drought-only plants. Immunoblot analyses revealed that drought strongly induced HSP60, two HSP23 isoforms (19 and 23 kDa), and 33 and 36 kDa dehydrin proteins. Proline treatments differentially modulated these stress-associated proteins, with moderate concentrations optimizing their accumulation, whereas higher doses amplified or suppressed specific responses depending on the protein examined. Notably, proline also altered protein expression patterns under non-stress conditions, indicating a regulatory or priming-like effect. These findings demonstrate that proline-mediated drought tolerance in pepper involves integrated osmotic regulation and stress-protein modulation, highlighting its potential as a concentration-dependent agronomic strategy to enhance crop resilience under water-limited conditions.

1. Introduction

Climate change driven by global warming, together with increasing drought frequency and extreme precipitation, significantly limits crop productivity. In arid and semi-arid regions, drought is a major abiotic constraint, as water constitutes 80–95% of plant biomass and its deficit disrupts key physiological processes (Kaur et al. 2024). In recent decades, agricultural water resources have declined due to increasing demand and enhanced evapotranspiration, and water availability is expected to become a major limiting factor for crop production across more than half of the world's arable land in the coming decades (IPCC 2023; Holzgreve et al. 2025).

Cellular water loss disrupts membrane integrity and metabolic processes, leading to increased electrolyte leakage and excessive production of reactive oxygen species (ROS), which cause lipid peroxidation, protein damage, impairment of photosynthesis, and ultimately cell death (Khan et al. 2025). To mitigate these effects, plants activate antioxidant systems and accumulate osmolytes such as proline, which functions both as an osmoprotectant and an effective ROS scavenger (Abdelaal et al. 2020; Abd-El-Aty et al. 2024). Proline accumulation also contributes to osmotic adjustment, stabilizes proteins and

membranes, and may regulate stress-associated proteins involved in tolerance mechanisms (Jurkonienė et al. 2023).

Among these proteins, heat shock proteins (HSPs) and dehydrins (DHNs) play critical roles in plant responses to drought stress. HSPs are induced under stress conditions and contribute to protein folding and protection, while their expression is often associated with stress tolerance in various plant species (ul Haq et al. 2019). Dehydrins, members of the late embryogenesis abundant (LEA) protein family, are highly responsive to dehydration and contribute to cellular protection through structural stabilization (Szlachtowska and Rurek 2023). Beyond its role as an osmoprotectant and ROS scavenger, proline has been suggested to contribute to stress tolerance by stabilizing proteins and membranes and by modulating the accumulation and functionality of stress-associated proteins, including heat shock proteins and dehydrins.

Given the increasing need to mitigate yield losses under drought conditions, the use of exogenous applications has gained considerable attention as an effective strategy to enhance plant tolerance. In this context, proline has been widely reported to improve plant performance under water deficit conditions (Kayak et al. 2022; Khan et al. 2025).

Pepper (*Capsicum annuum* L.) is one of the most economically important vegetable crops cultivated worldwide and is highly valued for its nutritional and commercial significance. However, pepper plants are particularly sensitive to water deficit conditions, and drought stress can severely reduce growth, physiological performance, fruit yield, and quality (Penella and Calatayud 2018). Due to its economic importance and susceptibility to drought, pepper represents a suitable model crop for investigating physiological and molecular mechanisms associated with drought tolerance and for evaluating potential stress mitigation strategies.

Given the increasing need to mitigate yield losses under drought conditions, the use of exogenous applications has gained considerable attention as an effective strategy to enhance plant tolerance. In this context, proline has been widely reported to improve plant performance under water deficit conditions (Kayak et al. 2022; Khan et al. 2025).

Therefore, the present study investigates the effects of exogenous proline application on the accumulation of key stress-associated proteins, namely heat shock proteins and dehydrins, in the *Capsicum annuum* L. cv. Yalova Çorbacı 12 under drought stress, aiming to elucidate protein-based mechanisms underlying proline-mediated drought tolerance.

2. Materials and Methods

2.1. Experimental condition

Commercial pepper seeds (*Capsicum annuum* L. cv. Yalova Çorbacı 12) were sown in peat-filled trays and transplanted at the 2–3 leaf stage into 17 × 18 cm pots containing soil, perlite, and peat (1:1:1). Plants were grown in a controlled greenhouse (25/18°C day/night, 60 ± 5% RH, 16/8 h light/dark) and fertilized weekly with NPK (7:7:7). At the 5–6 leaf stage, plants were divided into drought-stressed (10% PEG 6000) and non-stressed groups. Throughout the experiment, control plants were irrigated as needed to maintain soil moisture at field capacity, while drought-treated plants received an equivalent volume of 10% PEG-6000 solution corresponding to the irrigation volume applied to the control pots.

Proline (5, 10, and 20 mM) was applied as a foliar spray at 5-day intervals (days 0, 5, and 10). The experiment followed a randomized complete block design with three replicates (six plants each) and was terminated after 14 days.

2.2. Physiological parameters

Ion leakage was determined using three 1 cm leaf discs rinsed with deionized water and incubated in 10 mL deionized water at room temperature (100 rpm, 4 h). Initial electrical conductivity (EC_1) was measured (Mettler Toledo, SevenEasy S30, Columbus, OH, USA), followed by total conductivity (EC_2) after autoclaving at 121°C for 15 min and cooling. Ion leakage was calculated as $EC_1/EC_2 \times 100$ (İlik et al. 2018). Leaf relative water content (RWC, %) and turgor loss were determined according to Gulen and Eris (2003) using three replicates of three 1 cm leaf discs per treatment. Fresh weight (FW) was recorded immediately, turgid weight (TW) after 4 h in deionized water, and dry weight (DW) after drying at 70°C for 48 h. RWC and turgor loss were calculated using Equations 1 and 2 (Gulen and Eris 2003).

$$RWC (\%) = \frac{FW - DW}{TW - DW} \times 100 \quad 1$$

$$Loss\ of\ Turgor = \frac{TW - FW}{TW} \times 100 \quad 2$$

2.3. Proline content and protein analysis

Proline content was determined according to Bates et al. (1973). Leaf tissue (0.2 g) was extracted with 3% sulfosalicylic acid, and the supernatant reacted with acid ninhydrin and glacial acetic acid (100°C, 1 h). After cooling, proline was extracted with toluene, and absorbance was measured at 520 nm to calculate concentration from a standard curve.

Total soluble proteins (TSPs) were extracted from 0.25 g leaf tissue in borate buffer (pH 9.0) at 4°C, centrifuged (10000 rpm, 10 min), and quantified by the Bradford (1976) assay. Proteins (10 µg per lane) were separated by SDS-PAGE and visualized with Coomassie Brilliant Blue.

Membranes were probed with antibodies against HSP60 (mouse monoclonal; Sigma), HSP23 (rabbit polyclonal; Sigma), and dehydrin (polyclonal; AgriSera, AS07 206) (1:1500), and detected using an alkaline phosphatase-based colorimetric kit (ProtoBlot Western Blot AP Kit, Promega).

At harvest, plants were analysed for physiological and molecular parameters (n= 6). Data (mean ± SEM) were evaluated by two-way ANOVA to assess the effects of proline, drought, and their interaction, followed by Duncan's test ($P \leq 0.05$) using IBM SPSS Statistics 29. Prior to ANOVA, data were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively.

3. Results

3.1. Physiological parameters

Drought stress increased ion leakage from 9.3% to 15.0% in untreated plants (Table 1), whereas proline-treated plants exhibited lower values (11.1–12.4%). Two-way ANOVA revealed a significant effect of drought stress and a significant drought × proline interaction ($P < 0.001$), while the main effect of proline was not significant ($P = 0.140$).

RWC remained relatively stable in non-stressed plants, ranging from 81.8% to 83.1% across proline treatments (Table 1). Drought stress reduced RWC to 75.9% in untreated plants, whereas proline-treated plants maintained higher values ranging from 79.7% to 82.3%. Two-way ANOVA indicated that drought stress significantly affected RWC ($P < 0.001$). Proline application also exerted a significant main effect ($P = 0.006$). Moreover, the drought × proline interaction was significant ($P = 0.006$), indicating that the influence of proline on plant water status depended on the water regime.

Similarly, drought stress increased turgor loss from approximately 14–15% to 21.0% in untreated plants (Table 1). Proline treatment mitigated this effect, reducing turgor loss to 14.7–17.7%, with the lowest value recorded at 10 mM. Significant effects of drought stress, proline application, and their interaction were detected ($P < 0.001$).

Endogenous proline content responded strongly to drought stress. While non-stressed plants contained 1075–1535 µM g⁻¹ FW proline, drought-stressed untreated plants accumulated 7266 µM g⁻¹ FW (Table 1). Two-way ANOVA revealed significant effects of drought stress ($F = 2291.147$, $P < 0.001$), proline application ($F = 73.681$, $P < 0.001$), and their interaction ($F = 67.763$, $P < 0.001$), indicating that the influence of exogenous

proline on endogenous proline accumulation depended on the osmotic stress condition.

TSP content was reduced by drought stress, decreasing from 28.2–29.5 to 26.8–27.8 mg protein g⁻¹ FW (Table 1). Two-way ANOVA revealed significant effects of drought stress ($F=98.831$, $P<0.001$) and proline application ($F=8.874$, $P=0.001$) on TSP content. However, the drought $\times$ proline interaction was not significant ($F=0.490$, $P=0.694$), suggesting that the effect of proline was independent of the water regime.

Protein bands ranging from 19 to 70 kDa were detected, and their intensities varied among treatments (Figure 1A). The 70 kDa band was detected in all treatments and generally exhibited high intensity, although a noticeable reduction was observed in non-stressed plants treated with 10 and 20 mM proline. The 42 kDa band showed greater intensity in drought-stressed plants, particularly in the untreated and 20 mM proline groups, whereas lower intensity was observed at 5 and 10 mM. Under non-stressed conditions, its intensity was also enhanced at 20 mM proline.

The 36 and 33 kDa bands were present in all treatments. The 36 kDa band exhibited increased intensity at 5 mM proline in non-stressed plants and at 20 mM under drought stress, whereas the 33 kDa band showed enhanced accumulation in drought-stressed plants, especially at 0 and 20 mM proline.

The 28 and 23 kDa bands generally displayed higher intensities in non-stressed plants. In contrast, the 19 kDa band was reduced by drought stress; however, its intensity increased in drought-stressed plants receiving 10 or 20 mM proline.

3.2. Expression profiles of HSP 60, HSP23 and dehydrin proteins in response to drought stress and proline treatments

Immunoblot analyses revealed a 42 kDa HSP60 whose band intensity was markedly influenced by drought stress and proline treatments (Figure 1B). In non-stressed plants, HSP60 band intensity increased at 5 and 20 mM proline but declined at 10 mM relative to the control. Drought stress alone increased HSP60 band intensity; however, under drought conditions, it was lower at 5 and 10 mM proline and higher at 20 mM.

Two HSP23 isoforms (19 and 23 kDa) exhibited distinct band intensity patterns in response to drought stress and proline application (Figures 2A and B).

The 19 kDa isoform showed increased band intensity in response to proline treatment, with the highest intensity observed at 5 mM under both water regimes. Although band intensity declined at higher concentrations, it remained above control levels. Similarly, the 23 kDa isoform exhibited increased band intensity at 5 and 10 mM proline in non-stressed plants. Drought stress increased its band intensity, whereas exogenous proline partially attenuated this response, with the highest band intensity among proline-treated plants again detected at 5 mM.

Immunoblot analysis also identified two dehydrin bands (33 and 36 kDa), whose intensities varied according to drought stress and proline treatment (Figures 3A and B). The 33 kDa dehydrin showed increased band intensity in response to proline under non-stressed conditions, although band intensity gradually

Table 1. Effect of exogenous proline treatments on ion leakage, relative water content (RWC), turgor loss, proline and total soluble protein (TSP) content in pepper leaves under non-stress and drought stress conditions. Results are the means of three replicates $\pm$ SD ($P<0.05$). Means followed by different letters indicate significant differences among treatment combinations according to Duncan's multiple range test at $P<0.05$.

Groups	Proline Treatment	Ion Leakage (%)	RWC (%)	Turgor Loss (%)	Proline Content ($\mu\text{M g}^{-1}$ FW)	TSP (mg protein g ⁻¹ FW)
Non-stressed	0 mM	9.3 $\pm$ 0.2 d	82.8 $\pm$ 2.2 a	14.6 $\pm$ 0.4 c	1135.1 $\pm$ 70.8 f	28.2 $\pm$ 0.1 bc
	5 mM	12.1 $\pm$ 1.0 b	81.8 $\pm$ 1.5 a	15.0 $\pm$ 1.3 c	1326.9 $\pm$ 136.7 ef	28.8 $\pm$ 0.1 ab
	10 mM	10.9 $\pm$ 0.4 b-d	82.8 $\pm$ 0.7 a	14.3 $\pm$ 0.7 c	1075.0 $\pm$ 76.7 f	29.5 $\pm$ 0.5 a
	20 mM	10.0 $\pm$ 0.1 cd	83.1 $\pm$ 0.6 a	14.2 $\pm$ 0.4 c	1535.0 $\pm$ 70.2 e	29.1 $\pm$ 0.1 a
Drought-stressed	PEG+0 mM	15.0 $\pm$ 0.3 a	75.9 $\pm$ 2.9 b	21.0 $\pm$ 0.8 a	7266.2 $\pm$ 151.5 a	26.8 $\pm$ 0.0 e
	PEG+5 mM	11.1 $\pm$ 0.4 bc	79.7 $\pm$ 3.6 a	17.7 $\pm$ 1.0 b	4927.3 $\pm$ 143.8 c	27.3 $\pm$ 0.1 de
	PEG+10 mM	11.3 $\pm$ 0.6 bc	82.3 $\pm$ 1.1 a	14.7 $\pm$ 0.8 c	3631.9 $\pm$ 103.9 d	27.8 $\pm$ 0.2 cd
	PEG+20 mM	12.4 $\pm$ 0.8 b	80.0 $\pm$ 3.7 b	17.5 $\pm$ 0.1 b	5711.1 $\pm$ 92.2 b	27.2 $\pm$ 0.1 de

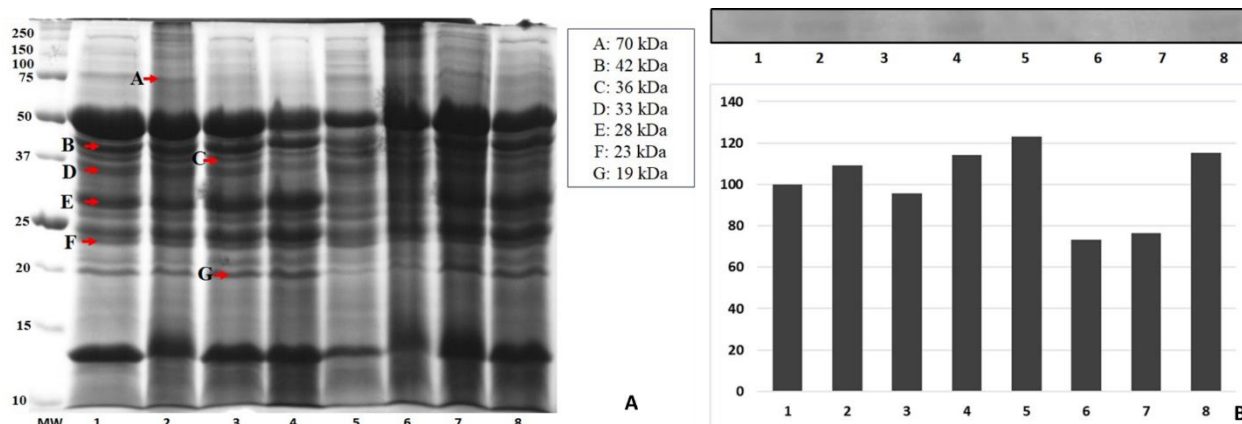


Figure 1. (A) Effects of proline treatments on SDS-PAGE protein profiles in pepper leaves under non-stress and drought stress conditions (MW: molecular weight marker, kDa). (B) Appearance of the 42 kDa HSP60 protein and its band intensity. 1: Control; 2: 5 mM; 3: 10 mM; 4: 20 mM; 5: PEG; 6: PEG + 5 mM; 7: PEG + 10 mM; 8: PEG + 20 mM.

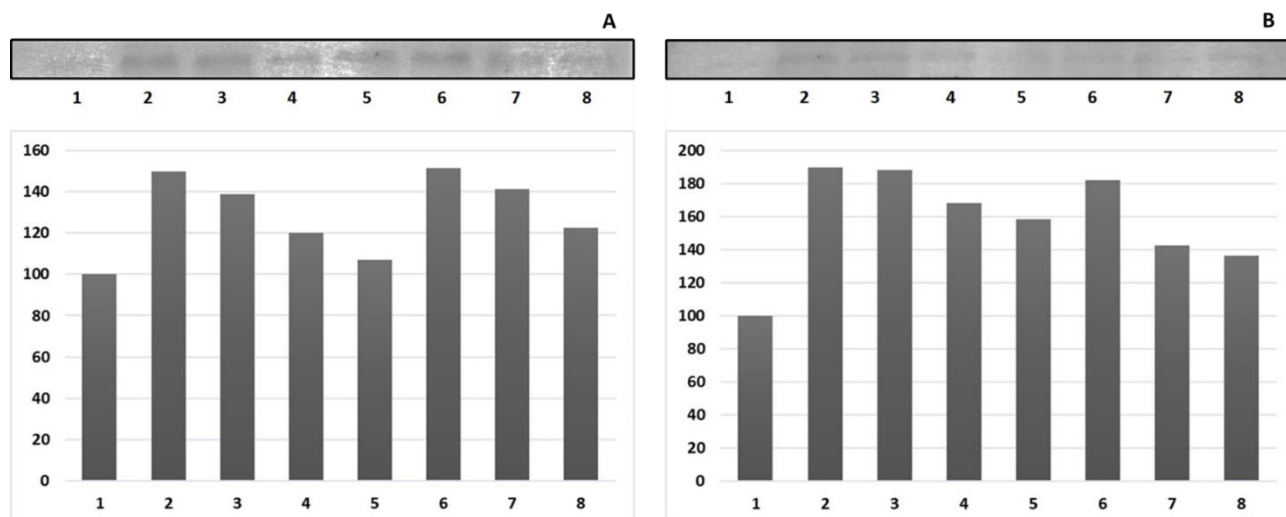


Figure 2. (A) Appearance of the 19 kDa and (B) 23 kDa HSP23 proteins and their band intensities in the leaves of pepper plants under non-stress and drought stress conditions with different concentrations of proline treatments. 1: Control; 2: 5 mM; 3: 10 mM; 4: 20 mM; 5: PEG; 6: PEG + 5 mM; 7: PEG + 10 mM; 8: PEG + 20 mM.

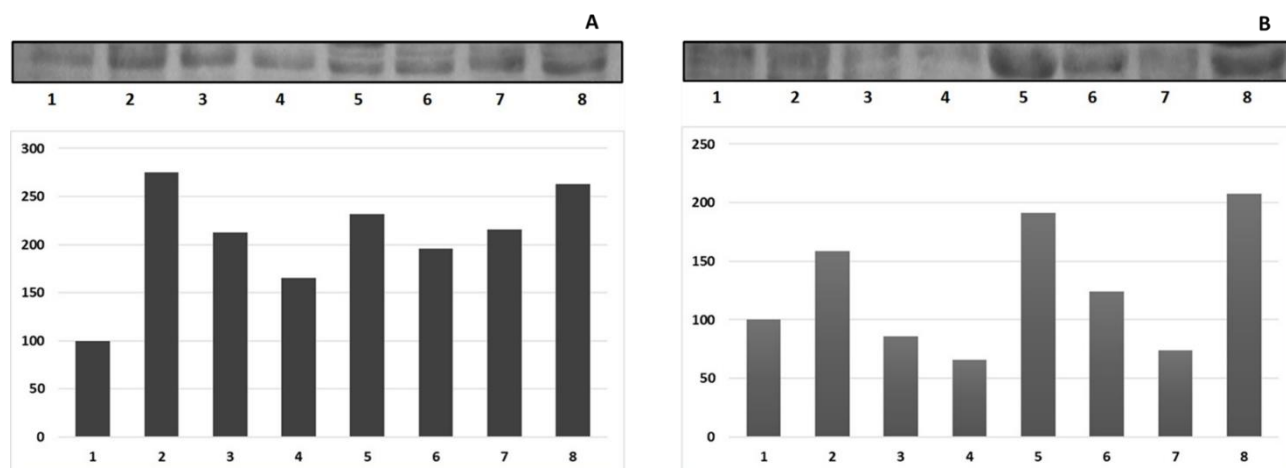


Figure 3. (A) Appearance of the 33 kDa and (B) 36 kDa dehydrin proteins and their band intensities in the leaves of pepper plants under non-stress and drought stress conditions with different concentrations of proline treatments. 1: Control; 2: 5 mM; 3: 10 mM; 4: 20 mM; 5: PEG; 6: PEG + 5 mM; 7: PEG + 10 mM; 8: PEG + 20 mM.

declined with increasing proline concentration. Drought stress approximately doubled its band intensity relative to the non-stressed control, and the highest intensity was observed at 20 mM proline. The 36 kDa dehydrin responded differently, showing maximum band intensity at 5 mM under non-stressed conditions but reduced intensity at higher concentrations. Under drought stress, band intensity increased in untreated plants and at 20 mM proline, whereas it was lower at 5 mM and reached its minimum at 10 mM.

4. Discussion

Drought stress markedly impaired membrane integrity, cellular water balance, and protein homeostasis in pepper plants, as reflected by increased ion leakage, reduced RWC, elevated turgor loss, and decreased TSP content. These responses are characteristic consequences of dehydration-induced oxidative stress, which destabilizes membrane lipids and accelerates protein denaturation and degradation (Abd-El-Aty et al. 2024; Khan et al. 2025). The significant drought $\times$ proline interactions

observed for ion leakage, RWC, turgor loss, and endogenous proline levels demonstrate that the effectiveness of exogenous proline was strongly dependent on the water regime. Under drought conditions, proline application, particularly at 5 and 10 mM, substantially reduced membrane damage and improved plant water status, indicating enhanced osmotic adjustment and cellular stabilization. These findings support the well-established role of proline in maintaining membrane integrity through phospholipid bilayer stabilization and protection of membrane-associated proteins (Jurkonienė et al. 2023; Khan et al. 2025).

The concentration-dependent responses observed here suggest that proline functions within a narrow optimal physiological window. While moderate concentrations improved osmotic balance and hydration status, the highest concentration (20 mM) appeared less effective, potentially due to metabolic constraints or feedback regulatory mechanisms. Emerging evidence indicates that excessive exogenous proline may disrupt redox homeostasis or stimulate proline catabolism through activation of proline dehydrogenase, resulting in regulatory

compensation rather than additional protection (Jurkonienė et al. 2023).

Interestingly, drought stress alone induced substantial endogenous proline accumulation, whereas exogenous proline application under drought resulted in comparatively lower endogenous proline levels. This response is consistent with feedback inhibition of proline biosynthesis and may involve the downregulation of P5CS and/or modulation of ProDH activity. Recent molecular studies indicate that exogenous proline supply is tightly coordinated with endogenous proline metabolism, highlighting its role within an integrated regulatory network rather than as a passively accumulating osmolyte (Abd-El-Aty et al. 2024).

Under non-stressed conditions, moderate increases in endogenous proline at 5 and 20 mM suggest metabolic incorporation of externally supplied proline, whereas the reduction observed at 10 mM further supports concentration-dependent regulation. The increased proline accumulation and improved physiological performance observed in the present study agree with the findings of Anjum et al. (2012), who reported that proline accumulation contributes to drought tolerance in pepper through osmotic adjustment and protection against stress-induced damage.

Drought significantly reduced TSP content, reflecting impaired protein synthesis and enhanced proteolysis under oxidative conditions. Although proline exerted a modest but significant main effect on TSP content, the absence of a significant drought $\times$ proline interaction suggests that its stabilizing influence on protein metabolism may operate independently of drought severity. Recent proteomic studies suggest that proline can function as a molecular shield, preserving enzyme conformation and preventing irreversible protein aggregation without necessarily stimulating de novo protein synthesis (Khan et al. 2025).

While these physiological responses indicate improved cellular protection and water balance, the protein expression profiles provide further insight into the molecular mechanisms underlying proline-mediated drought tolerance.

At the molecular level, drought stress strongly induced the accumulation of stress-associated proteins, including HSP60, HSP23 isoforms, and dehydrins, confirming their important role in drought adaptation. Heat shock proteins function as molecular chaperones that prevent protein aggregation, facilitate the refolding of denatured proteins, and maintain proteostasis under stress conditions. The increased accumulation of HSP60 under drought is consistent with previous reports demonstrating enhanced chaperonin activity in response to dehydration-induced protein misfolding (ul Haq et al. 2019). Recent systems-level analyses further suggest that HSP networks integrate ROS signalling and osmotic cues, forming a central component of drought-responsive regulatory pathways (Jurkonienė et al. 2023).

The strong induction of HSP23 isoforms under drought is likewise consistent with their established role in stabilizing partially unfolded proteins and protecting photosynthetic structures under stress conditions. Similar drought-induced expression of small heat shock proteins has been reported in pepper, where proteins such as CaHSP16.4, CaHSP18.1a, and CaHSP25.9 contribute to enhanced stress tolerance through protection of cellular structures and maintenance of physiological functions (Feng et al. 2019; Huang et al. 2019; Liu et al. 2021). Together, these findings support the view that enhanced HSP

accumulation represents a key component of the drought response in pepper.

The differential modulation of HSPs by exogenous proline suggests a close interaction between osmoprotection and chaperone-mediated defense mechanisms. Moderate proline concentrations (5–10 mM) reduced HSP60 abundance relative to drought-stressed plants without proline treatment, indicating that improved protein stability may have lowered the requirement for chaperone upregulation. In contrast, 20 mM proline markedly enhanced HSP60 accumulation, suggesting possible signalling cross-talk between proline metabolism and HSF-mediated transcriptional activation. Similarly, the pronounced accumulation of HSP23 isoforms at 5 mM proline indicates that moderate osmolyte supplementation may optimize stress signalling and protein protection without imposing excessive metabolic costs. Such priming-like enhancement of sHSP expression at moderate osmoprotectant concentrations has also been reported in recent drought studies (Abd-El-Aty et al. 2024). Collectively, these findings suggest that exogenous proline not only contributes to osmotic adjustment but also modulates stress-responsive protein networks involved in drought adaptation.

Dehydrins (33 and 36 kDa) also exhibited marked drought responsiveness, confirming their protective roles in membrane stabilization and protein protection during cellular dehydration. As intrinsically disordered LEA proteins, dehydrins interact with membranes, ions, and denatured proteins to preserve cellular structure under water deficit conditions. Recent structural studies have emphasized their multifunctional protective capacity under both osmotic and oxidative stress (Szlachowska and Rurek 2023). The increased dehydrin accumulation observed in drought-stressed plants is consistent with previous reports describing the involvement of dehydrins in drought tolerance in pepper. Jing et al. (2016) identified several dehydrin genes in *Capsicum annuum* that are induced under abiotic stress conditions, while Meng et al. (2021) demonstrated that CaDHN3 contributes to drought tolerance through improved osmotic adjustment and reduced oxidative damage.

The concentration-dependent modulation of dehydrin accumulation by proline further highlights the complex regulatory interplay between osmolyte supplementation and stress-protein networks. Moderate proline concentrations reduced dehydrin abundance relative to drought-only plants, likely reflecting improved cellular hydration and reduced stress intensity. In contrast, 20 mM proline enhanced the accumulation of the 33 kDa dehydrin, suggesting synergistic activation of stress-responsive pathways at higher concentrations. These observations support emerging concepts that osmolytes can function not only as protectants but also as signalling molecules within integrated stress-response networks (Jurkonienė et al. 2023; Khan et al. 2025).

Under non-stress conditions, proline altered TSP content and modulated the accumulation of HSP60, HSP23, and dehydrin proteins, indicating that proline acts as a metabolic regulator even in the absence of drought stress. The enhanced accumulation of specific sHSPs and dehydrins at moderate concentrations suggests a mild activation of protective pathways consistent with stress priming. Recent studies describe proline-induced priming as a preparatory mechanism that enhances cellular readiness for subsequent stress exposure without imposing significant physiological penalties (Abd-El-Aty et al. 2024).

Overall, the present study demonstrates that proline-mediated drought tolerance in pepper involves the coordinated

regulation of both physiological and molecular defense mechanisms. Improvements in membrane stability, water status, and protein homeostasis were accompanied by concentration-dependent changes in the accumulation of HSPs and dehydrins, highlighting the interconnected nature of osmotic adjustment and stress-protein responses. These findings support the view that proline functions not only as an osmoprotectant but also as a signalling modulator that influences multiple components of the drought-response network.

Although the present study focused primarily on physiological and protein-based responses, future research should evaluate growth-related traits such as fresh and dry biomass accumulation to determine whether the observed protective effects translate into improved plant growth and productivity under drought conditions. In addition, field-based studies are needed to assess the practical applicability and economic feasibility of exogenous proline treatments by considering application costs, yield responses, and potential economic returns. Such studies would further clarify the potential of optimized proline application as a management strategy for enhancing drought resilience in pepper cultivation.

5. Conclusion

The present study demonstrates that exogenous proline modulates drought tolerance in pepper through integrated physiological and protein-based mechanisms. Drought stress severely impaired membrane integrity, water status, and protein stability, whereas proline application mitigated these effects in a concentration-dependent manner. Moderate proline concentrations, especially 5 mM, generally provided the most favorable physiological and molecular responses by maintaining cellular hydration, reducing membrane damage, and regulating the accumulation of stress-associated proteins, including HSP60, HSP23 isoforms, and dehydrins.

The findings further reveal that exogenous proline does not simply accumulate additively within plant tissues but interacts dynamically with endogenous proline metabolism, indicating feedback regulation under osmotic stress. Moreover, the modulation of stress-responsive proteins under both stress and non-stress conditions suggests that proline functions not only as an osmoprotectant but also as a metabolic and signalling regulator capable of fine-tuning protein quality-control systems.

These results provide mechanistic insight into how exogenous proline coordinates physiological stabilization with stress-protein networks in a concentration-dependent manner, thereby contributing to drought tolerance. The findings provide a physiological and molecular basis for the potential use of proline as an agronomic tool to enhance drought resilience in pepper and other horticultural crops.

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