

## Research Article

# Proteomic Responses of Tissue-Cultured Date Palm (*Phoenix dactylifera* L. cv. Barhi) to Salinity, Drought, and Heavy Metal Stress

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**Abstract** | Date palm (*Phoenix dactylifera* L. cv. Barhi) is affected by abiotic stresses that influence its physiology and yield. This study assessed effects of salinity, drought and lead toxicity on leaf protein profiles of tissue cultured propagated seedlings using SDS–PAGE. A total of 51 protein bands were recorded, with number per treatment ranging from 5 to 8 in control and under combined stress (150 mM NaCl + 21-day irrigation interval + Pb 100 mg kg<sup>-1</sup>) respectively. Molecular weights ranged from approximately 11 to 281 kDa. Two concentric bands were observed: a high molecular weight band (255–281 kDa) and an approximately 47 kDa band, authenticating uniform genetic origin of seedlings. Stress treatments induced discrete variations: high salinity (150 mM NaCl), prolonged drought (21-day irrigation), and Pb at 100 mg kg<sup>-1</sup> each produced 7 bands, while combined stresses meaningfully increased band numbers to 8. This shows enhanced protein synthesis and the expression of genes related to stress. The control group, subjected to minor stress treatments (7-day irrigation, Pb 50 mg kg<sup>-1</sup>), showed differences. Severe and combined stresses accounted for the highest proteomic shifts. These results can be translated into an investigation that shows that abiotic stresses notably alter the proteomic structure of date palm leaves, and also signify electrophoretic protein profiling as a reliable biochemical tool for assessing stress overview in this economically valuable cultivar of date palm.

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## Introduction

The date palm (*Phoenix dactylifera* L.) is one of the primitive cultivated fruit trees and a basis of agricultural systems in arid and semi-arid. The date palm (*Phoenix dactylifera* L.) is one of the primitive cultivated fruit trees and a basis of agricultural

systems in arid and semi-arid climates (Yuniar *et al.*, 2024). In addition to its nutritional and cultural value, it sustains rural livelihoods and makes a substantial economic impact on date-producing nations, especially those in the region of the Middle East and North Africa (Elhoumaizi *et al.*, 2021; FAO, 2022). Historically, Iraq was among the leading producers

across the globe, the date palm producers, but has experienced a sudden decline in both cultivated area and productivity over the recent past, a trend closely linked with environmental degradation and causes of climate change (Qureshi *et al.*, 2021; Al-Mahmoudi *et al.*, 2023).

Abiotic stresses such as salinity, drought and heavy metal toxicity are among most serious constraints limiting date palm production of sustainable basis. Soil salinity has become major issue in Iraq as a result of poor-quality irrigation water and insufficient drainage, which leads to reduction in plant vigor and fruit yield (Qureshi *et al.*, 2021; Efrani *et al.*, 2024). Similarly, drought stress caused by declining water resources and more frequent dry spells negatively impacts physiological activities such as regulation of stomata, photosynthesis and assimilate partitioning (Hussain *et al.*, 2022). Heavy metal stress, particularly caused by lead (Pb), has also become an emerging issue in irrigated agriculture, where industrial waste and polluted water sources contribute to accumulation of toxic heavy metals in soils. Lead toxicity inhibits the enzymatic activities, induces oxidative stress and alters protein metabolism pathways in plants (Shahid *et al.*, 2014; Syarbiah *et al.*, 2024). Collectively, these stresses pose a major threat to survival, productivity and genetic stability of date palm cultivars. To enhance resilience against stress in plants, it is important to understand how plants respond to stress at molecular level is therefore essential for developing strategies (Odeh *et al.*, 2024). Proteins are direct products of gene expression; provide critical insights into adaptive responses. Variations in protein expression patterns under stress conditions can disclose activation of defense mechanisms, stress caused gene regulation and physiological adjustments (Zhang *et al.*, 2024). Among available tools, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) remains a robust, cost-effective, and reproducible method for assessing qualitative and quantitative variation in total soluble proteins. It is extensively used to characterize stress responses, evaluate genetic diversity and identify biochemical markers in diverse crops (Ranjan *et al.*, 2013; Taain *et al.*, 2021).

Even though date palm is an economically important perennial fruit plant, systematic studies associating abiotic stresses to proteomic changes remain limited. Previous research has primarily emphasized on growth, yield and physiological attributes, with

relatively few attempts to analyze protein banding patterns in relation to stress resilience (Taain *et al.*, 2021). Moreover, the proteomic responses of tissue culture propagated cultivars, particularly Barhi, are largely unexplored, despite their commercial significance and susceptibility to stress (Hamza and Almansour, 2024).

The present study aims to fulfill this knowledge gap by evaluating the effects of salinity, drought, and lead toxicity on protein banding patterns of tissue cultured date palm cv. Barhi using SDS-PAGE. By characterizing the number, molecular weight distribution, and treatment-specific protein bands, the study aims to provide biochemical evidence of stress-induced proteomic shifts. The findings will contribute to a better understanding of molecular responses in date palm and highlight the potential of protein profiling as a diagnostic tool for stress tolerance and genetic stability in this species.

#### *Plant material and experimental design*

Tissue cultured seedlings of date palm (*Phoenix dactylifera* L. cv. Barhi) were obtained from the Date Palm Research Center, Agriculture College, Basrah University, Iraq. Uniform seedlings, 8–10 months old, were transplanted into plastic pots (25 cm diameter, 10 kg soil capacity) containing a standardized substrate mixture of loamy soil, peat moss, and sand (2:1:1, v/v). Plants were maintained in a greenhouse under controlled conditions (28 ± 2 °C; natural photoperiod; 60–65% relative humidity).

The study was conducted in a completely randomized design (CRD) with three replicates per treatment, each replicate consisting of five seedlings. Stress treatments were imposed as follows:

- Control (C): Irrigation with tap water every 7 days.
- Drought stress (D): Irrigation withheld for 21 days.
- Salinity stress (S): Irrigation with 150 mM NaCl solution.
- Lead stress (Pb): Soil application of Pb(NO<sub>3</sub>)<sub>2</sub> at 100 mg kg<sup>-1</sup>.
- Combined stress (S + D + Pb): Application of salinity, drought, and Pb simultaneously.

Stress treatments continued for 8 weeks, after which fully expanded young leaves were collected, immediately frozen in liquid nitrogen, and stored at -80 °C until protein extraction.

**Protein extraction**

Total soluble proteins were extracted from 0.5 g of frozen leaf tissue following the protocol of [Taain et al. \(2021\)](#), with modifications. Tissues were ground in liquid nitrogen and homogenized in 2 mL of extraction buffer (50 mM Tris-HCl, pH 7.5; 10 mM EDTA; 1% w/v polyvinylpyrrolidone [PVP]; 5 mM dithiothreitol [DTT]). The homogenized mixture was centrifuged at 12,000 × g for 20 min at 4 °C and supernatant was collected as crude protein. Protein concentrations were estimated as explained by [Bradford, 1976](#), with bovine serum albumin (BSA) as the standard. Recent studies confirm the reliability of this approach for quantifying soluble proteins in stressed date palm tissues ([Zhang et al., 2024](#)).

**SDS–page analysis**

Protein profiling was conducted using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) as performed by [Laemmli \(1970\)](#). For each sample, 30 µg of protein was combined with loading buffer, denatured at 95 °C for 5 minutes and loaded onto gels containing 12% resolving layer and a 5% stacking layer. Electrophoresis was done at 120 V for approximately 2 hours using a Mini-PROTEAN® system (Bio-Rad, USA).

After electrophoretic separation, gels were stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 for 3 hours and subsequently destained in a methanol: acetic acid: water solution (4:1:5, v/v/v) until protein bands were distinctly visible. Gel images were captured using a Bio-Rad Gel Doc™ XR+ system, and molecular weights of bands were determined relative to a pre-stained protein ladder (10–250 kDa).

**Data analysis**

Protein bands were scored manually as present (1) or absent (0) across treatments to construct a binary data matrix. The number of bands, their molecular weight distribution, and relative mobility (Rf values) were compared across stress conditions. Descriptive statistics were used to summarize banding patterns. One-way analysis of variance (ANOVA) was performed in SPSS v.25 (IBM, USA), and treatment means were separated using Tukey’s HSD test at *p* < 0.05.

**Results**

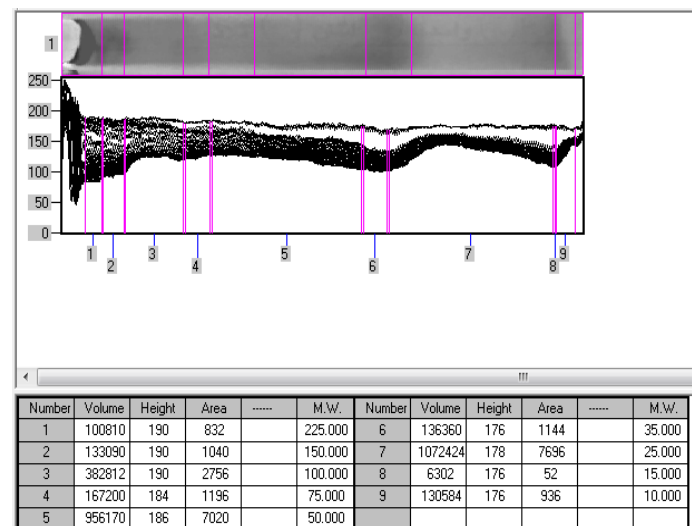
**Total number of protein bands**

A total of 51 protein bands were detected across all treatments, with molecular weights ranging from

11.2 to 281.2 kDa ([Table 1](#); [Figure 1](#)). The number of bands per treatment ranged between 5 and 8. The combined stress treatment (salinity + drought + Pb) showed the highest number of bands (8), while the control treatment exhibited the lowest (5).

**Table 1:** *The transaction codes and their type of impact.*

| Impact type      | Treatments  | Treatments number |
|------------------|---|-------------------|
| -                | Marker  | 1                 |
| Individual       | Salts 25 mM As a control treatment                                  | 2                 |
| Individual       | Salts 150 mM  | 3                 |
| Individual       | Irrigation every 7 days   | 4                 |
| Individual       | Irrigation every 21 days  | 5                 |
| Individual       | Pb 50 mgkg <sup>-1</sup>  | 6                 |
| Individual       | Pb 100 mgkg <sup>-1</sup>   | 7                 |
| Combina-<br>tion | Salts 150 mM + Irrigation every 21 days + Pb 100 mgkg <sup>-1</sup> | 8                 |
| Combina-<br>tion | Salts 150 mM + Irrigation every 21 days + Pb 50 mgkg <sup>-1</sup>  | 9                 |



**Figure 1:** *Some specifications of protein bundles on polyacrylamide gel for marker.*

**Conserved protein bands across treatments**

Two protein bands were consistently expressed in all treatments. The first was a high molecular weight band ranging from 255.0 to 281.2 kDa. The second conserved band appeared in the 46.8–47.6 kDa range, though its relative position varied, being ranked as the third, fourth, or fifth band depending on treatment ([Figure 2](#)). The presence of these conserved bands indicates that they are stable proteins expressed irrespective of stress conditions.

**Stress-specific protein bands**

Distinctive bands were observed under different stress treatments. Drought stress induced an

additional band in the lower molecular weight range, while lead treatment produced unique bands in the mid-range molecular weights (60–75 kDa). Salinity stress generated bands of smaller size (~20 kDa). The combined stress treatment resulted in both high- and low-molecular-weight bands not present in the individual stresses.

*Molecular weight distribution of bands*

Protein bands were distributed across a wide molecular weight range (11.2–281.2 kDa). High molecular weight proteins (above 200 kDa) were present in all treatments but were most prominent in the control and drought treatments. Mid-range proteins (40–75 kDa) were more frequent in the Pb and combined stress treatments. Low molecular weight proteins (<30 kDa) were primarily detected under salinity and combined stress conditions (Figure 2).

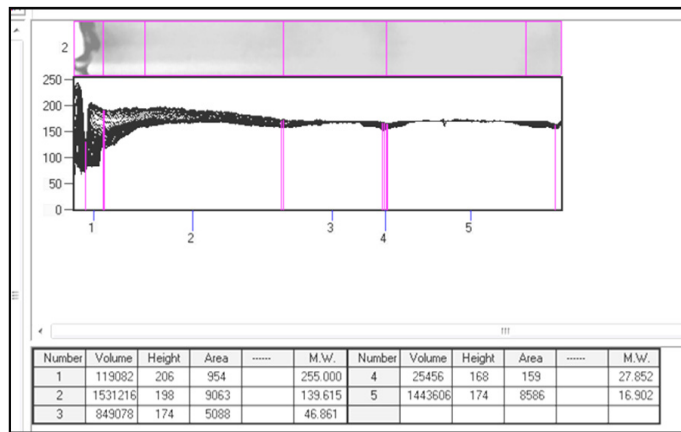


Figure 2: Some specifications of protein bands on polyacrylamide gel for comparison treatment.

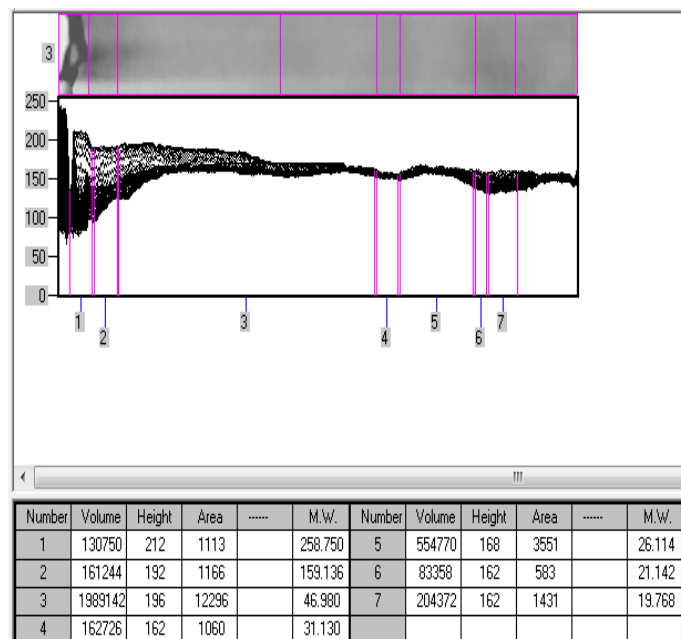


Figure 3: Some specifications of protein bands on polyacrylamide gel for 150 mM salinity treatment.

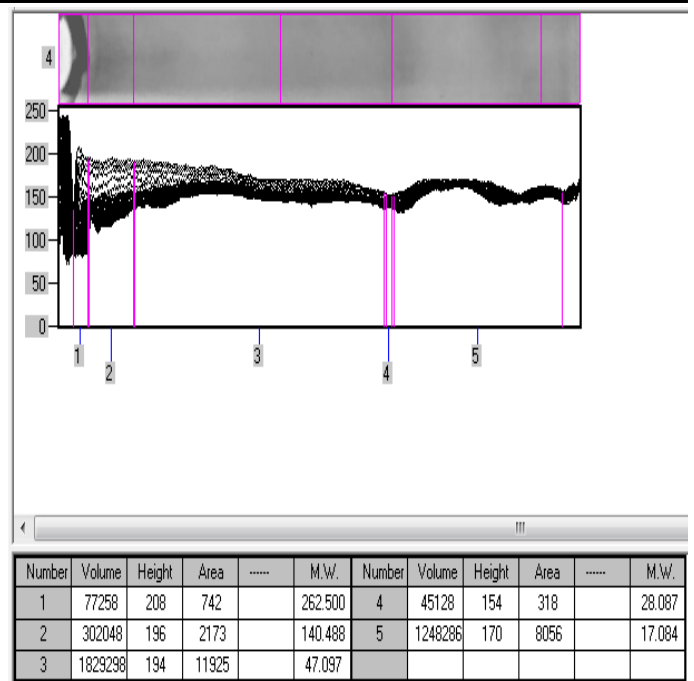


Figure 4: Some specifications of protein bundles on polyacrylamide gel for irrigation treatment every 7 days.

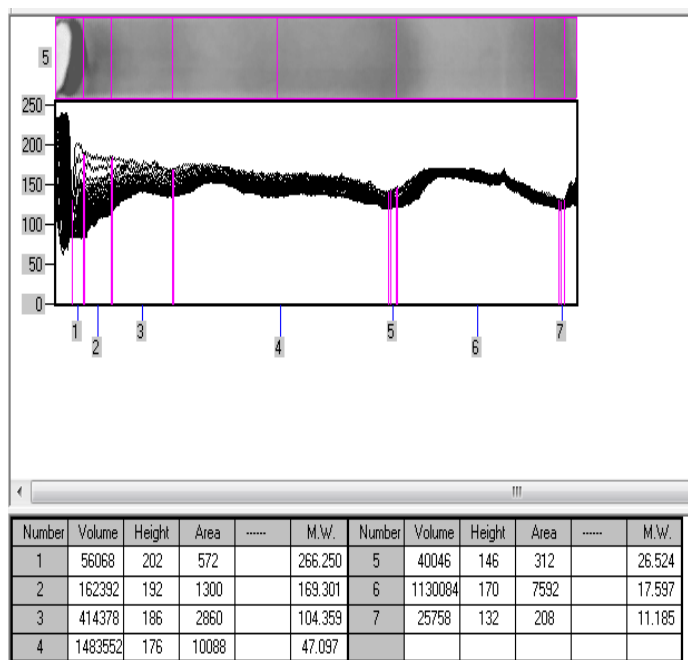


Figure 5: Some specifications of protein bundles on polyacrylamide gel for irrigation treatment every 21 days.

*Shared and unique protein profiles*

While all treatments shared at least two common bands, several bands were exclusive to individual stresses. Five protein bands were present in seedlings exposed to three conditions: control, irrigation at 7-day intervals, and Pb at 50 mg kg<sup>-1</sup>. Compared to single-stress settings, notably combined stress treatments consistently generated more distinct protein bands.

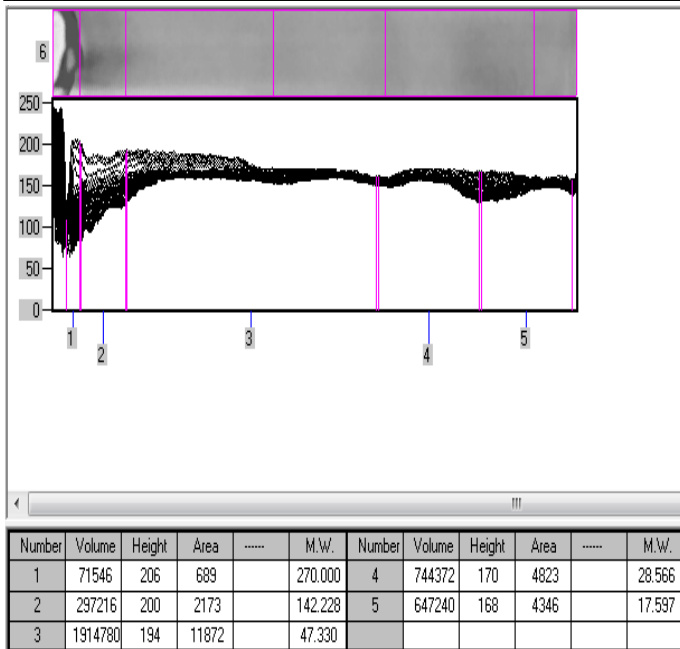


Figure 6: Some specifications of protein bands on polyacrylamide gel for lead treatment at a concentration of 50 mg kg<sup>-1</sup>.

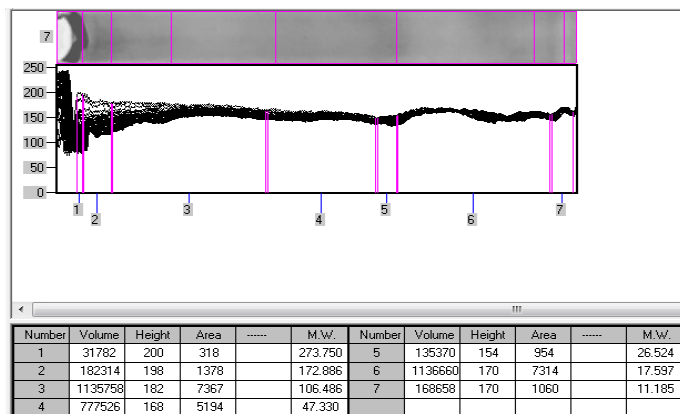


Figure 7: Some specifications of protein bands on polyacrylamide gel for lead treatment at a concentration of 100 mg kg<sup>-1</sup>.

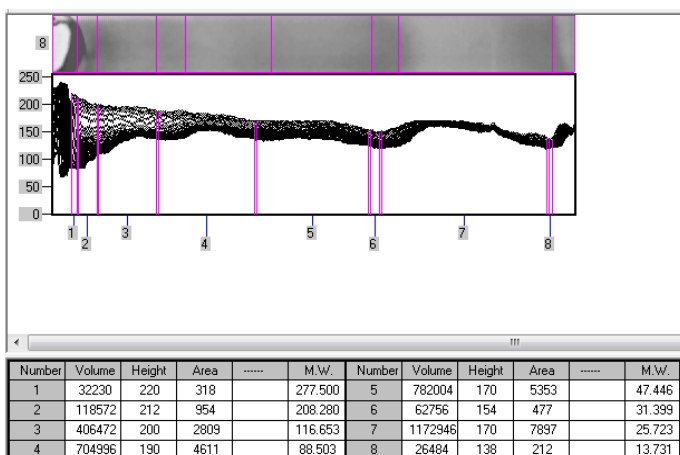


Figure 8: Some specifications of protein bands on polyacrylamide gel for the triple interference treatment 150 mM + irrigation every 21 days + lead 100 mg kg<sup>-1</sup>.

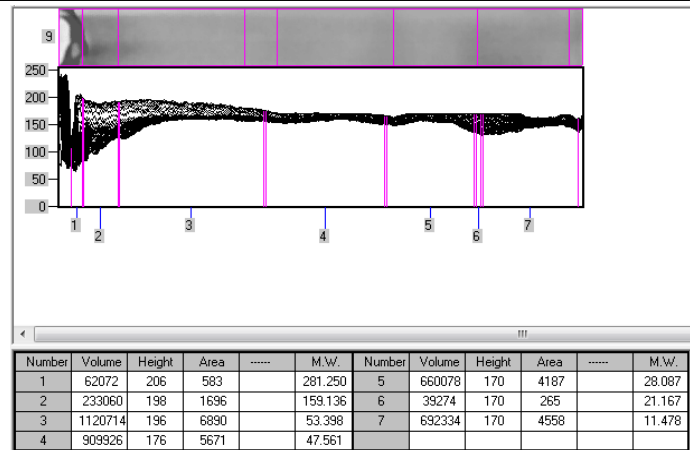


Figure 9: Some specifications of protein bands on polyacrylamide gel for the triple interference treatment 150 mM + irrigation every 21 days + lead 50 mg kg<sup>-1</sup>.



Figure 10: Number and locations of protein bundles and their molecular weights for.

## Discussion

As shown in our findings of this study, demonstrated that abiotic stresses induced marked alterations in the soluble protein profiles of *Phoenix dactylifera* cv. Barhi, as revealed by SDS-PAGE analysis. A total of 51 protein bands were recorded across treatments, with variation in both the number and molecular weights of bands depending on the type of stress applied (Ali *et al.*, 2024; Ahmad *et al.*, 2025). Control plants showed the fewest bands, whereas the combined stress treatment produced the most, indicating that exposure to multiple stresses activated additional proteins not observed under individual stress conditions. This expansion of protein expression is consistent with reports that plants subjected to environmental constraints undergo extensive proteomic reprogramming to enhance tolerance and survival (Raza *et al.*, 2023; Zhang *et al.*, 2024).

Despite these treatment-specific differences, two notable bands were consistently expressed in all seedlings. One was a high-molecular-weight band between 255 and 281 kDa, and the other was in the range of 46–47 kDa, though its relative position varied slightly among treatments. The stable occurrence of these proteins suggests their essential role in primary metabolism or cellular structure, and their persistence across treatments also indicates genetic uniformity of the tissue-propagated seedlings. Similar stability of conserved protein bands has been reported in clonal populations of date palm and other perennial crops, where they are considered signatures of genetic fidelity (Al-Qurainy *et al.*, 2019; Maryam *et al.*, 2023).

In contrast, distinctive proteins were expressed under specific stresses. weight proteins appeared as a result of the drought; these could be dehydrins or late embryogenesis abundant proteins that are normally linked to desiccation tolerance. Lead exposure induced unique mid-range proteins (60–75 kDa), consistent with proteins involved in metal detoxification and antioxidant defense, while salinity generated small molecular weight proteins likely linked to osmotic adjustment and ion homeostasis. These patterns confirm that different abiotic stresses mobilize distinct sets of stress-responsive proteins, a phenomenon also observed in proteomic studies of cereals and palms (Hussain *et al.*, 2022; Rao *et al.*, 2021).

The combined stress treatment was particularly notable, as it stimulates to produce greatest number and diversity of protein bands, including several that were absent in single stress was induced to plants (Maryam *et al.*, 2025b). This proposes a synergistic effect in which simultaneous stresses cause unique molecular responses unpredictable from individual treatments. Recent proteomics research has displayed that plants facing multiple stresses simultaneously often activate novel defense mechanisms, particularly those related to energy metabolism and redox regulation, which help buffer against cumulative damage (Raza *et al.*, 2023; Maryam *et al.*, 2025a). Our observation of additional proteins in the combined treatment consequently highlights the plasticity of date palm proteome and also highlights the importance of studying stress combinations to more accurately represent field conditions.

Overall, our findings provide strong evidence that

SDS–PAGE protein profiling can serve as a practical approach for identifying stress induced biochemical changes in date palm. The conserved bands confirm genetic stability of the tissue cultured Barhi seedlings, While we note these are the appearance of stress-specific bands highlights significant biochemical markers of tolerance. Surprisingly, proteins in 20–40 kDa range under drought and salinity, and those in the 60–75 kDa range under lead stress, permit further exploration as candidate biomarkers for stress resilience. Coupling such biochemical markers with advanced proteomic and genomic approaches could accelerate the identification and selection of stress-tolerant genotypes in date palm, considered a vital phase in maintaining output as environmental difficulties grow.

## Conclusion

The present study revealed that abiotic stresses, including drought, salinity and lead exposure, induce clear changes in the protein expression patterns of tissue cultured date palm cv. Barhi seedlings. While conserved bands authenticated genetic stability of the propagated seedlings, the appearance of stress specific proteins indicated distinct adaptive responses to individual and combined stresses. Significantly, combined stress conditions triggered highest diversity of protein bands, suggesting activation of additional molecular pathways not observed under single stresses. These findings focus the potential of SDS–PAGE profiling as a rapid and reliable approach for identifying biochemical markers of stress responses in date palm. Such markers can provide valuable insights into tolerance mechanisms and lay the groundwork for integrating proteomic tools into breeding strategies aimed at developing resilient cultivars capable of sustaining productivity under multiple environmental constraints.

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## Novelty Statement

Date palm seedlings cv. Barhi. exhibit changes in protein expression because of abiotic stressors like salinity, lead (Pb) exposure, and drought. The SDS–PAGE results revealed the appearance of specific proteins, showing adaptive responses to both individual

and combined stresses.

## Author's Contribution

**Ahmed Z. Resan:** Conceived and designed the research and conducted the experiments and collected the data.

**Ahmed Y. Lafta:** Assisted in data analysis and interpretation and contributed to methodology refinement and laboratory supervision.

**Harith M. Al-Tamimi:** Drafted the manuscript.

All authors read and approved the final version of the manuscript.

### Generative AI and AI-assisted technology statement

The authors declare that no Generative AI was used in the creation of this manuscript.

### Conflict of interest

The authors have declared no conflict of interest.

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