MDPI
agronomy an Open Access Journal by MDPI
IECAG 2024 Conference

Two-Dimensional Electrophoresis Highlights Proteomic Shifts in Grapevines (*Vitis vinifera* L.) Exposed to Drought Under Field Conditions

Catarina Estêvão[1], Ana Margarida Sequeira[2], Lénia Rodrigues[1*], Catarina Campos[1], Mónica Marques[1], Lourenço Charters[3], Amândio Rodrigues[3], Ana Carina Neto[3], Rui Flores[3], Augusto Peixe[4], Maria João Cabrita[4], Hélia Cardoso[5]

[1]MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE — Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal; [2]Escola de Ciências e Tecnologia, Universidade de Évora, Colégio Luis António Verney, Rua Romão Ramalho, 59, 7000-671 Évora, Portugal; [3]Esporão S.A., Herdade do Esporão, Apartado 31, 7200-999 Reguengos de Monsaraz, Portugal; [4]MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE — Global Change and Sustainability Institute, Escola de Ciências e Tecnologia, Departamento de Fitotecnia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal; [5]MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE — Global Change and Sustainability Institute, Escola de Ciências e Tecnologia, Departamento de Biologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

*Corresponding author: **liar@uevora.pt**

Grapevine (*Vitis vinifera* L.) is one of the oldest and most economically significant fruit crops in the world.

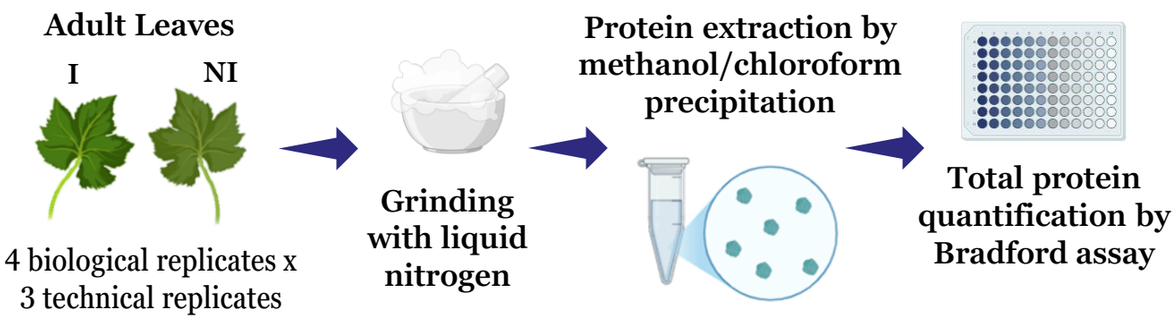
It **adapts to stress** by altering gene expression, leading to **proteome changes** that drive metabolic and physiological adjustments.

Proteomic studies reveal key stress-response proteins and their roles in metabolic pathways, helping to understand plant resilience.

This study applied **two-dimensional electrophoresis (2-DE)** to evaluate the protein profile of **leaves of *Vitis vinifera* cv. 'Touriga Nacional'**, from **irrigated (I)** and **non-irrigated (NI)** vines, and identify differentially expressed proteins.

Methodology

Sample processing and Total protein extraction

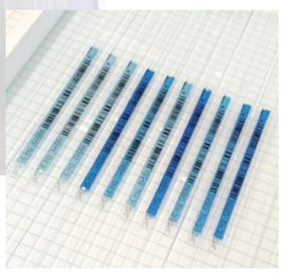


2-DE analysis of protein extracts

1 **First dimension:** Isoelectric focusing (IEF)

Separates the proteins according to their isoelectric point

Immobilized pH gradients (IPG) strips (7 cm, 3–11 pH gradient) were rehydrated for 16h with 80µg of protein



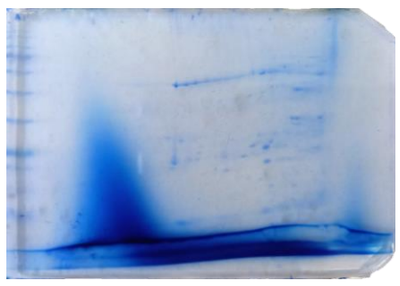
IEF was performed in Multiphor II system at 12°C, applying 200 V (1 Vh), 200-3500 V (until reaching 2800 Vh), 3500 V (until reaching 10 000 Vh), and 3500 V (until reaching 5200Vh)

2 **Second Dimension:** Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Separates the proteins according to their molecular weight

The SDS-PAGE was carried out in 14% acrylamide gels, at 130V, at room temperature

3 **Detection and Visualization of Proteins**



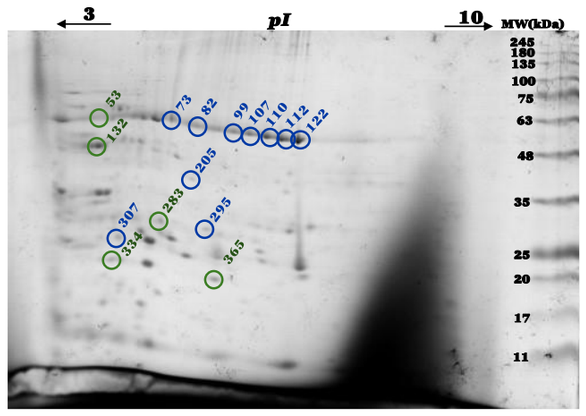
Proteins were stained with Coomassie Brilliant blue G-250

The gels were scanned with ImageScanner III (GE Healthcare)

The 2-DE images were analyzed using SameSpots, TotalLab programme

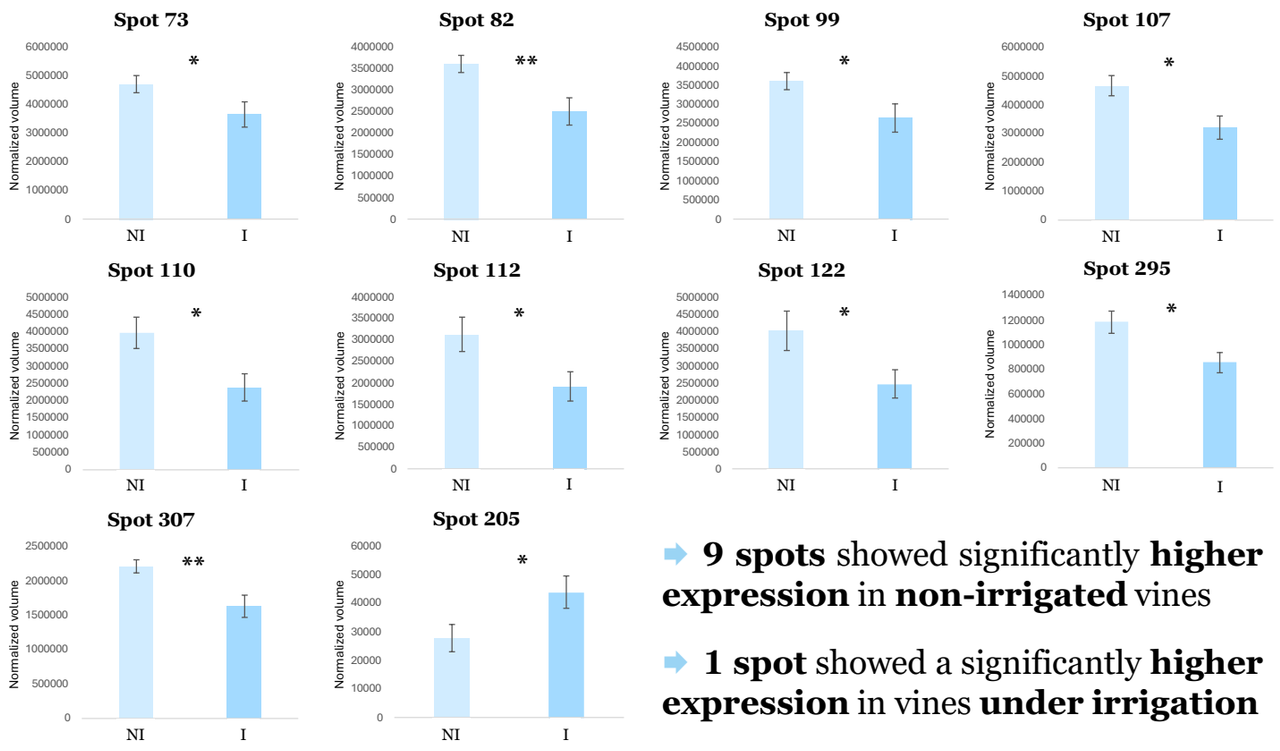
Results and Conclusions

The comparison of the proteomic profiles of I and NI samples uncovered differences in protein expression



Representative 2-DE proteomic profile: sample NI, biological replicate 3, technical replicate 3

- **47 protein spots were considered for analysis**
- **10 spots were identified as differentially expressed (marked in blue)**
- **5 spots showed a trend toward differential expression (marked in green)**



- **9 spots** showed significantly **higher expression** in **non-irrigated** vines
- **1 spot** showed a significantly **higher expression** in vines **under irrigation**

Proteins were not identified by mass spectrometry; however, potential matches were inferred by comparing apparent molecular weights with published data on proteomic responses to drought-stress

Spots	Spot apparent molecular weight	Behaviour	Possible proteins according to literature
73, 82, 99, 107, 110, 112, 122	~ 60 kDa	Higher expression under drought stress	HSP70 (65-75 kDa) [1]; ATP synthase CF1 beta subunit (61 kDa) [1,2]; Glyceraldehyde-3-phosphate dehydrogenase (59 kDa) [1,2]; Catalase subunits (55-60 kDa) [3,4]
205	~ 40 kDa	Lower expression under drought stress	LHC chlorophyll binding proteins (30-40 kDa) [5]
295, 307	~ 30 kDa	Higher expression under drought stress	HSP26 (26 kDa) [6,7]; Glutathione S-transferase (30 kDa) [6,7]; Ascorbate peroxidase (27-29 kDa) [1]; Superoxide dismutase (20-30 kDa) [7]

[1] https://doi.org/10.1007/s10725-020-00586-4 [2] https://doi.org/10.3389/fpls.2021.749184 [3] https://doi.org/10.1186/1471-2229-13-49 [4] https://doi.org/10.1042/BCJ20240247 [5] https://doi.org/10.3390/agronomy10050680 [6] https://doi.org/10.1016/j.jplph.2016.11.016 [7] https://doi.org/10.1016/j.plaphy.2021.08.010

The work is financed by the project BioGrapeSustain. Vine&Wine – SubProject Proposal – Project 81. Agenda Mobilizadora: Driving Sustainable Growth Through Smart Innovation (Reference C644866286-00000011) and by National Funds through FCT under the Project UI/BD/153509/2022 and Project UIDB/05183/2020.