



OPEN Physiological and proteomic analysis reveal high recovery capacity of quinoa (*Chenopodium quinoa* Willd.) after progressive drought stress

Rahma Goussi^{1,2}, Marcello Manfredi^{3,4}, Emilio Marengo², Simone Cantamessa², Roberto Barbato⁵ & Arafet Manaa¹✉

Quinoa, a resilient crop adapted to marginal environments, offers a promising alternative under water-limited conditions. To gain new insights into its drought tolerance, we combined physiological analyses with quantitative proteomics under progressive drought (1–2 weeks) and subsequent recovery (2 weeks). Drought stress induced significant reductions in biomass (56%) and relative water content (46%) after two weeks, with leaf osmotic potential decreasing by up to 48%. However, plants exhibited strong recovery in these parameters upon rehydration. Proteomic analysis identified 114 differentially abundant proteins between drought treatments and control. Proteins involved in cell defense and cytoskeleton organization including S-adenosylmethionine synthetase, caffeoyl-CoA O-methyltransferase, actin, and Exocyst complex component EXO84A, showed an increase under short-term drought conditions. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) increased during prolonged drought and further during recovery, while heat shock proteins (HSP90) accumulated under stress and declined after rewatering, supporting protein stability and oxidative defense. Multiple ribulose biphosphate carboxylase large and small subunits (RuBisCO) showed increased abundance under drought and further increased during recovery, while oxygen-evolving enhancer proteins (OEE1, OEE2) accumulated early (D1W), helping to stabilize PSII under stress. Interestingly, key proteins involved in amino acid metabolism, including ACT domain-containing protein (ACR11), glutamine synthetase, and Ferredoxin-dependent glutamate synthase (Fd-GOGAT), were identified as regulators of nitrogen assimilation and oxidative stress regulation. Betaine aldehyde dehydrogenase was decreased under stress and increased during recovery, reflecting osmoprotectant accumulation. This study, integrating physiological and proteomic data, demonstrates that quinoa deploys immediate protective mechanisms while activating adaptive processes related to stress memory and recovery-associated metabolic changes, highlighting its resilience under water deficit.

Keywords Drought stress, Recovery capacity, Comparative proteomics, SWATH-MS, Quinoa

Drought is considered as one of the most significant abiotic stresses, particularly in arid and semi-arid regions, where plants are regularly subjected to water scarcity and extended periods of drought, affecting plant growth, and ultimately lead to reduced crop yields¹. Moreover, the rising frequency and intensity of drought events, intensified by climate change, pose a serious threat to global food security and the ability to meet the needs of a rapidly growing population, estimated to reach around 10 billion people by 2050². To develop agricultural

¹Laboratory of Extremophile Plants, Centre of Biotechnology of Borj Cedria, B.P. 901, 2050 Hammam-Lif, Tunisia. ²Dipartimento Di Scienze E Innovazione Tecnologica, Università del Piemonte Orientale, Viale Teresa Michel 11, 15121 Alessandria, Italy. ³Department of Translational Medicine, Università del Piemonte Orientale, Novara, Italy. ⁴Department of Translational Medicine, Center for Translational Research On Autoimmune and Allergic Diseases, University of Piemonte Orientale, Novara, Italy. ⁵Department of Sustainable Development and Ecologica Transition, University of Eastern Piedmont Amadeo Avogadro, 13100 Vercelli, Italy. ✉email: arafet.manaa@cbbc.rnrt.tn; manaaarafet@gmail.com

solutions for feeding the growing global population, halophytes offer a promising source of resilient crops well-adapted to harsh climatic conditions such as water deficit³. One notable example is quinoa (*Chenopodium quinoa* Willd.), which was recognized by the FAO in 1996 as promising crop to address global nutrition challenges due to its exceptional nutritional value and health benefits⁴.

Quinoa is a pseudocereal and known as an important food source in the Andean region for thousands of years^{5,6}. Quinoa is a facultative halophyte well adapted to a broad range of agro-ecological conditions including marginal soil^{7,8} and highly resistant to a wide range of abiotic stresses, including salinity^{9,10}, drought¹¹, Jacobson et al.¹², frost¹³, and cold¹⁴. As gluten free seeds, quinoa has recently become a popular healthy food due to its quality of proteins with a balanced essential amino acid profile, dietary fiber, minerals, vitamins and good quality fats^{15–17}. Due to these exceptional nutritional properties, 2013 was declared the "International Year of Quinoa" to emphasize this alternative crop's potential to improve food security and promote sustainable agriculture¹⁸. Following the IYQ, quinoa has been widely used as model crop for understanding drought tolerance in halophytes^{11,19,20}. Most of the drought adaptation mechanisms of quinoa can be summarized as follows: (i) Distinct morphological traits, such as : extensively ramified root system to enhanced water uptake, reduction of leaf area through leaf dropping, and the presence of calcium oxalate-rich epidermal bladder cells that help reduce transpiration^{14,21}. (ii) Low osmotic potential, low turgid weight/dry weight ratio, low elasticity and an ability to maintain positive turgor even at low leaf water potentials²². (iii) The flexibility of quinoa's photosynthetic apparatus, attributed to its high PSII efficiency, along with the structural integrity of its chloroplasts^{11,23}. (iv) The high overall antioxidant capacity²⁴.

Plant drought tolerance is a complex trait controlled at cellular and whole-organism levels, and become more intricate as the effect of drought stress vary with intensity, duration, and phenological stage¹. The physiological, biochemical and genetic mechanisms associated with plant drought tolerance are evident not only during drought stress period but also during the recovery process after re-watering²⁵. Moreover, the capacity for recovery after rewatering strongly depends on the plant species and the intensity/duration of the imposed stress, with quinoa being one of the most resilient demonstrating a remarkable ability to withstand drought episodes at different development stages^{11,20,26}. The majority of conducted studies on quinoa have focused on morphological and physiological mechanisms, while only a few have addressed the molecular mechanisms, identifying key proteins and/or genes that play crucial roles in drought resistance.

The main objective of the present investigation is to study the proteome variation in quinoa plants subjected to two consecutive water stress periods and subsequent recovery episodes. With the intention of achieving this goal, a quantitative proteomics analysis by SWATH mass spectrometry (Sequential Window Acquisition of all Theoretical ion spectra) was performed to identify and quantify the proteins extracted from quinoa leaves of plants cultivated in different conditions.

The insights gained from exploring the quinoa proteome will contribute to the existing knowledge on the complexity of drought resistance and serve as a basis for breeding programs aimed at developing quinoa varieties suited for specific conditions, such as water scarcity.

Material and methods

Plant growth conditions

Seeds of *Chenopodium quinoa* (var. Red Faro), originating from southern Chile and provided by the USDA International Seed Bank, were used in this study. Red Faro was selected because it is widely recognized as one of the most resistant quinoa genotypes, capable of producing high biomass even under elevated salinity (500 mM NaCl)⁹. In addition, this variety exhibits efficient and robust physiological, biochemical, and anatomical mechanisms that enable it to cope with severe drought conditions¹¹. Seeds were surface sterilized for 5 min by soaking in a 20% (v/v) sodium hypochlorite solution, followed by thorough rinsing with distilled water (up to 5 times). Afterward, quinoa seeds were sown in 0.6-L plastic pots at a density of 4 seeds per pot, each filled with limono-sandy soil enriched with: 0.25 meq 100 g⁻¹ of Na⁺, 0.95 meq 100 g⁻¹ of K⁺, 0.65 meq 100 g⁻¹ of Ca²⁺, and 0.05 meq 100 g⁻¹ of Cl⁻, as well as 0.24 g kg⁻¹ of P₂O₅ and 0.45 g kg⁻¹ of total nitrogen (N). The electrical conductivity and pH of the aqueous extract (1/10) were 0.04 dS/m, and 6.55, respectively. The field capacity (FC) of soil was 11%, according to Bouyoucos (1983). During the first week of germination, seedlings were irrigated with distilled water and then with the full-strength Hoagland solution²⁷ containing the following macronutrients (mM): 0.5 Ca(NO₃)₂, 1.25 KNO₃, 0.5 MgSO₄, 0.625 KH₂PO₄, and micronutrients (ppm): 2.8 Fe, 0.55 Mn, 0.06 Zn, 0.06 Cu, 0.32 B and 0.02 Mo. Plant culture was conducted in a climate-controlled greenhouse with temperature of 25/18 °C and relative humidity of 60/70% day/night, respectively.

Emerged seedlings of uniform size were subjected to a 10-days acclimation period, during which soil was maintained at 95% field capacity (FC). When the plants reached the five-true leaf stage, they were subjected to different water treatments (Fig. 1) as following:

- Control : Well-irrigated plants maintained at 95% field capacity (FC).
- D1W: Plants exposed to water deficit at 15% FC for 1 week.
- D2W: Plants exposed to water deficit at 15% FC for 2 weeks.
- R1W: Plants previously subjected to D1W, re-watered at 95% FC for 2 weeks.
- R2W: Plants previously subjected to D2W, re-watered at 95% FC for 2 weeks.

Irrigation was performed every 2 days (regular weighing) to restore the soil moisture at 95 or 15% FC. During the drought period, in order to maintain the same quantity of nutrients received by all treated or untreated plants, 2 g of slow release Osmocote (10N:11P:18 K) was added to the soil mixture at the beginning of drought treatment. Different harvests were performed for each treatment (Fig. 1). Fresh leaf samples from each plant were immediately frozen in liquid nitrogen and stored at -80 °C until the proteomic analysis was performed.

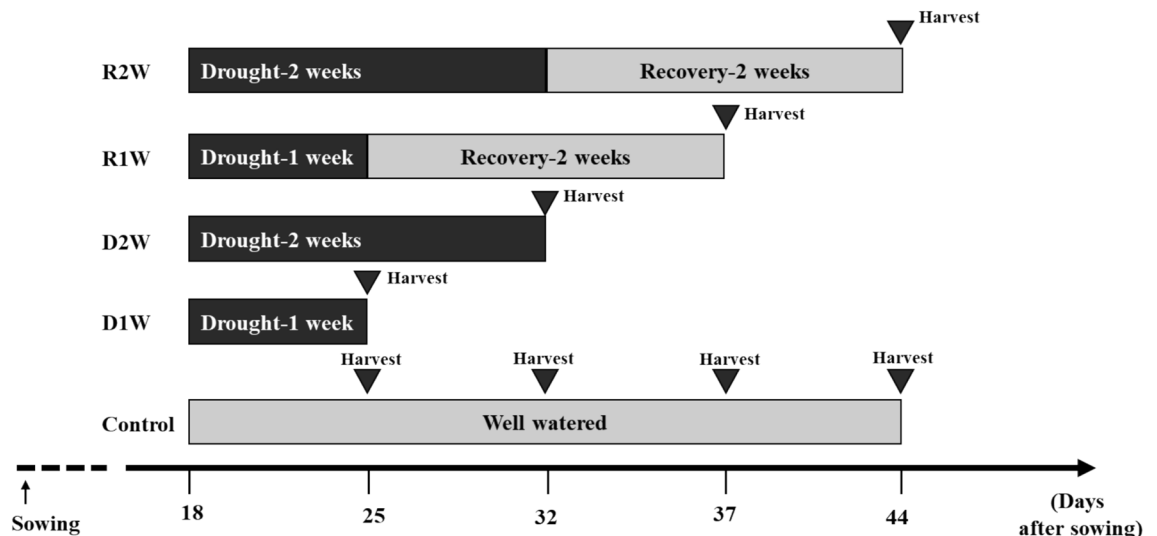


Fig. 1. Experimental design and timeline of quinoa cultivation under control and drought stress conditions. Drought stress was imposed for either one week (D1W) or two weeks (D2W), followed by a subsequent two-week recovery period (R1W and R2W, respectively). Four harvests were conducted at 7, 14, 21, and 28 days after the initiation of stress treatment.

Growth and water content measurement

During each harvest, plant growth was assessed by measuring the fresh weight (FW) and dry weight (DW) of the plants. Dry weight was determined after desiccating the plant material at 70 °C for 3 days. Turgid weight (TW) was measured by soaking leaves in distilled water in test tubes for 12 h at room temperature. After soaking, leaves were gently blotted with tissue paper to remove surface moisture before weighing.

The total dry matter content (TDM) and relative water content (RWC) of fully expanded leaves and was calculated using the following equations:

$$\text{RWC (\%)} = [(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100$$

$$\text{TDM (\%)} = (\text{DW} / \text{FW}) \times 100$$

Leaf osmotic potential

The leaf osmotic potential (Ψ_s) was measured according to Nobel²⁸. Quinoa leaves were harvested, sectioned into small fragments, transferred into Eppendorf tubes perforated with four small holes, and directly immersed in liquid nitrogen to promote cell rupture and facilitate subsequent extraction of cell sap. After being individually encased in a second intact Eppendorf tube, the samples were kept at room temperature for approximately 20 min to thaw before being pressed. The collected tissue sap was analyzed for (ψ_s) estimation. Osmolarity was assessed with a vapour pressure osmometer (Wescor 5500; Inc., UT, USA) and converted from mosmoles kg^{-1} to MPa according to the Van't Hoff equation as following:

$\Psi_s = -R T M$, where R represents the universal gas constant, T stands for the temperature (K) and M is the osmolality.

Proteins extraction and quantification

Leaf total proteins were extracted using a phenol extraction procedure according to Faurobert et al.²⁹. Leaves were frozen in liquid nitrogen, ground to a fine powder, suspended in 3 × volumes of an extraction buffer (700 mM sucrose, 500 mM Tris (pH 8), 100 mM KCl, 2% (v/v) β -mercaptoethanol, 2 mM PMSF, pH 8.5) and incubated for 10 min on ice; afterwards, an equal volume of Tris-saturated phenol was added. The samples were shaken for 10 min at room temperature and then centrifuged (5525 × g for 10 min at 4 °C) to separate the phenolic and the aqueous phase. The phenolic phase was recovered and re-extracted with the same volume of extraction buffer. Subsequently centrifugation was repeated and 5 volumes of precipitation solution (0.1 M ammonium acetate in methanol) was added to the recovered phenol phase. Proteins were allowed to precipitate at −20 °C overnight. After centrifugation (5525 × g for 10 min at 4 °C), the protein pellet was washed three times with the precipitation solution and once with acetone. Each washing step was followed by 5 min of centrifugation as described above. After drying under vacuum, the pellet was resuspended in lysis buffer (9 M Urea, 4% (w/v) CHAPS, 0.5% (v/v) Triton X-100, 20 mM DTT, 1.2% (v/v) pharmalytes pH 3–10) and protein concentration was measured according to modified Bradford assay³⁰.

Protein digestion, desalting, mass spectrometric analysis

Protein digestion and desalting

Protein digestion was performed according to the protocol described by Cordara et al.³¹, with modifications from Goussi et al.³². Proteins at a concentration of 0.5 mg/mL in denaturing buffer were reduced with 10 mM DTT (at 37 °C for 30 min) and alkylated with 20 mM iodoacetamide (at room temperature for 30 min in the dark). To maintain trypsin activity, the urea concentration was diluted up to 1 M by adding 50 mM Tris-HCl pH 8.0. Protein digestion was performed by adding Trypsin/Lys-C Mix (Promega, WI, USA) to obtain a final protein-to-protease ratio of 25:1 (w/w), followed by overnight incubation at 37 °C. Trifluoroacetic acid was added to a final concentration of 0.5% (v/v) to facilitate completeness of tryptic digestion process. To remove the insoluble material, the sample was centrifuged at 15000 × g for 10 min. Solid-phase extraction was then used for peptide desalting, following the method described by Guo and Kristal³³. To the resulting eluates, approximately 1,500 femtomoles of a synthetic heavy peptide internal standard (Cellmano Biotech, Hefei, China) were added, followed by lyophilization. The dried peptides were reconstituted in 30 µL of LC-MS/MS mobile phase A (water with 0.1% formic acid) and then analyzed by LC-MS/MS.

Mass spectrometry analysis

LC-MS/MS analyses were performed as described by Hellmann et al.³⁴, with modifications from Albanese et al.³⁵, using a micro-LC Eksigent Technologies (Dublin, USA) system which included a micro LC200 Eksigent pump with 5–50 µL flow module and a programmable autosampler CTC PAL with a Peltier unit (1–45 °C). The stationary phase consisted of a Halo Fused C18 column (0.5 × 100 mm, 2.7 µm, Eksigent Technologies, Dublin, USA). The mobile phase was a mixture of 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B), eluting at a flow-rate of 15.0 µL min⁻¹. An injection volume of 4.0 µL was used, with the gradient increasing the concentration of solvent B from 2 to 40% over 30 min. The oven temperature was maintained at 40 °C. The LC system was interfaced with a 5600+ Triple TOF™ system (AB Sciex, Concord, Canada) equipped with DuoSpray™ Ion Source and CDS (Calibrant Delivery System).

As previously described by Gillet et al.³⁶ with modification from Goussi et al.³², two different mass spectrometric acquisition workflows were adopted: (1) Data Dependent Acquisition (DDA) mode for proteins identification, and (2) Data Independent Acquisition (DIA) SWATH (Sequential Window Acquisition of all Theoretical fragment ion spectra) mode, for proteins quantification. The peptide profiling was performed using a mass range of 100–1600 Da (TOF scan with an accumulation time of 0.25 s), followed by a MS/MS product ion scan range 200–1250 Da (accumulation time of 5.0 ms) with the abundance threshold set at 30 cps (35 candidate ions can be monitored per cycle). The ion source parameters in electrospray positive mode were set as follows: curtain gas (N₂) at 25 psig, nebulizer gas (GAS1) at 25 psig, and (GAS2) at 20 psig, ion spray floating voltage (ISFV) at 5000 V, source temperature at 450 °C, and declustering potential at 25 V. Tryptic-digested samples, used to generate the SWATH-MS spectral library in DDA mode, were subsequently subjected to cyclic DIA of mass spectra with a 25 Da window width, following the methods described by Venable et al.³⁷ and Gillet et al.³⁶. The mass spectrometer was operated to perform a 50 ms survey scan (TOF-MS), followed by MS/MS experiments on all precursors. These latter were achieved in a cyclic manner using an accumulation time of 40 ms per 25 Da SWATH (36 total SWATHs) for a total cycle time of 17,408 s. The ions were fragmented in the collision cell during each MS/MS experiment using rolling collision energy. DIA analysis was conducted on four replicates for each sample. All MS data were acquired with Analyst TF1.7 (AB Sciex, Concord, Canada) and the Paragon algorithm³⁵. The following sample parameters were set: trypsin as the digestion enzyme, carbamidomethylation for cysteine alkylation and no special factors. The processing parameters were set to “Biological modification”.

The Data-Dependant Acquisition (DDA) were searched for protein identification, through an ID search, using the UniProt Knowledgebase (UniProtKB) database (<https://www.uniprot.org/>). Only protein IDs with a Protein Pilot unused score of at least 1.3 (equivalent to a 95% confidence interval) were accepted as the cutoff threshold, along with an estimated local false discovery rate (FDR) of no more than 1%. Proteins with fold changes ≥ 1.5 or ≤ 0.66 with adjusted *p*-values ≤ 0.05 were considered present at significantly different between the samples.

Data Availability: The protein identification generated and/or analysed during the current study are available in Table 1S.

Statistical analysis

Each experiment was performed using three independent replicates. The data obtained from all parameters are presented as the mean value ± SE (Standard Error). Statistical analysis was performed using One-Way ANOVA and Duncan's multiple range test for each parameter at *P* < 0.05, using OriginPro software (version 9.0 SR2; Northampton MA01060 USA).

Results and discussion

Physiological response to drought stress

Drought-induced water scarcity significantly affects the productivity and quality of major agricultural crops by limiting water availability, which can markedly impair plant growth and reduce yields³⁸. In response to water deficit, plants exhibit a wide array of complex changes at morphological, physiological, biochemical, and molecular levels^{1,24,39–41}. Plant growth and biomass yield, relative water content (RWC), and leaf water potential are classical indicators used to evaluate plant tolerance to abiotic stress, particularly drought^{42–44}. In our study, drought stress induced a significant reduction in biomass production, as assessed by dry weight (DW) (Fig. 2a, Table 1). This reduction was approximately 17% after one week of treatment and became more pronounced, reaching 56% as compared to the control, when the drought period was extended to two weeks (D2W). Additionally, Fig. 2b, showed that TDM was maintained under short-term drought (D1W) and

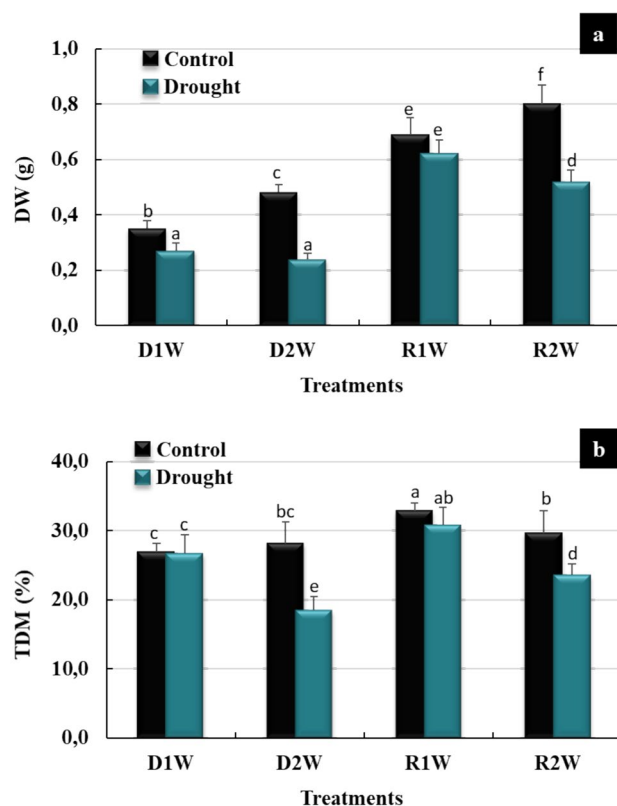


Fig. 2. (a) Total dry weight (DW) and (b) Total tissue dry matter content (TDM, %) of quinoa plants subjected to drought stress for one week (D1W) or two weeks (D2W), followed by a subsequent two-week recovery period (R1W and R2W, respectively). Data represent means of six replicates $\pm$ SE. Mean values with similar letters are not different at P -value < 0.05 according to Duncan's multiple range test at 95%. Blue bars represent drought treatments, while black bars represent the respective controls.

	D1W	D2W	R1W	R2W
DW (%)	17 $\pm$ 4.1 c	56 $\pm$ 5.2 a	7 $\pm$ 0.7 d	35 $\pm$ 4.4 b
RWC (%)	21 $\pm$ 2.2 b	46 $\pm$ 5.1 a	2 $\pm$ 0.8 c	26 $\pm$ 3.4 b
Ψ (Mpa)	-1,03 $\pm$ a	-1,31 $\pm$ c	-1,02 $\pm$ a	-1,12 $\pm$ b
Ψ (%)	28 $\pm$ 2.4 b	42 $\pm$ 4.3 a	3 $\pm$ 1.1 c	6 $\pm$ 2.2 c

Table 1. Percentage reduction (%) in dry weight (DW), relative water content (RWC) and leaf osmotic potential (Ψ) compared with control in quinoa plants subjected to drought stress for one week (D1W) or two weeks (D2W), followed by a subsequent recovery period of two-week (R1W and R2W, respectively). Data are means of 6 replicates $\pm$ SE. Means with similar letters are not different at $P < 0.05$ according to Duncan's multiple range test at 95%.

during initial recovery (R1W). However, prolonged drought (D2W) reduced TDM by 34%, and this reduction partially persisted even after recovery (R2W). Similarly, relative water content (RWC) decreased under drought conditions, with a significant reduction of 46% observed after two weeks of stress (D2W) (Table 1). However, following two-week recovery period, both DW and RWC were significantly restored in plants subjected to the one-week drought treatment (D1W), indicating that quinoa can partially restore biomass production after short-term drought stress. This aligns with numerous previous studies indicating rapid water potential recovery in various species after drought^{45,46} and in other quinoa varieties^{24,47}.

Leaf osmotic potential can serve as a key indicator of a plant's drought resistance and its capacity for recovery following water stress⁴⁸. In addition, drought tolerance is commonly characterized by a reduction in osmotic potential and the ability to sustain positive turgor pressure under conditions of water deficit. In our study, drought treatment resulted in a decrease in leaf osmotic potential by 28% and 48% after one and two weeks of water deficit, respectively (Table 1). However, this decline was gradually recovered upon rehydration, with osmotic potential values restored to levels comparable to those of the control. The observed recovery indicates that osmotic potential in quinoa leaves can be partially restored upon rehydration, which may play a role in the

plant's drought tolerance. Similar patterns of leaf osmotic adjustment and high capacity of recovery in quinoa varieties have been reported in previous studies^{19,49}.

However, the physiological characterization of the above basic parameters remains insufficient to fully elucidate the mechanisms underlying drought tolerance in quinoa. To gain deeper insight, we employed a quantitative proteomic approach using SWATH mass spectrometry to investigate proteome-level changes in response to drought stress and subsequent recovery.

Overview of leaf quantitative proteomic analysis under drought stress

The SWATH-MS method has been previously used to investigate protein variations under diverse stress conditions across multiple plant species⁵⁰. This label-free quantitative proteomic approach has proven effective in identifying differentially expressed proteins involved in responses to drought³², salinity⁵¹, and nutrient stress⁵², thereby contributing to a better understanding of stress tolerance mechanisms. These previous reports provided a detailed methodology and highlighted the potential of label-free SWATH-MS proteomics in plant research, emphasizing its high precision, reproducibility, and ability to detect low-abundance proteins, features that are particularly valuable for studying stress responses at the proteome scale. In the present study, this approach enabled us to assess both the extent of drought-induced changes in protein expression and the variability in proteomic profiles during rehydration. In fact, the relative quantification of leaf proteome revealed about 398 proteins (Table 1S), among them we were able to quantify 114 proteins showing a significant difference (with fold changes ≥ 1.5 or ≤ 0.66 and p -values ≤ 0.05) between drought treatments, as compared to control (Table 2).

As shown in Fig. 3a, 61 proteins exhibited significant changes in abundance (either increase or decrease) after one week of drought stress (Control vs. D1W), whereas 46 proteins were differentially abundant following two weeks of drought (Control vs. D2W). Interestingly, following the rewatering phase, the number of differentially abundant proteins increased substantially. Specifically, 96 proteins showed altered abundance one week after rewatering (Control vs. R1W), and 97 proteins were identified after the recovery from two weeks of drought (Control vs. R2W). These results indicate a dynamic proteomic response during the recovery phase, which may be associated with processes related to stress response, repair, and physiological adjustment. The principal component analysis (PCA) based on fold-change values of the differentially expressed proteins (Fig. 3c, d) clearly separated the treatments according to drought treatment and duration (Fig. 3d). PC1 and PC2 explained 62.18% and 27.67% of the total variance, respectively, accounting for 89.85% of the overall variability. Drought-stressed plants (D1W and D2W) were distinctly separated from rewatered plants (R1W and R2W) along PC1, indicating substantial proteomic alterations induced by water deficit and subsequent recovery. Furthermore, a clear separation between 1-week and 2-week treatments was observed along PC2 for both drought and recovery conditions, suggesting time-dependent changes in protein abundance.

The 114 proteins identified as common across all treatments were classified into seven functional categories (Fig. 3b). The largest groups were associated with "Energy and carbon metabolism" (28%) and "Photosynthesis" (18%), underlining the central role of these processes in the plant's response to drought stress and recovery. The remaining proteins were classified into five additional categories including: Cell wall reinforcement and cytoskeleton remodeling (9%), ROS detoxification and Heat Shock Proteins (17%), Amino acid and Protein biosynthesis (15%), DNA/ RNA assembly and structure (9%), and a mixed class called "miscellaneous" (4%), which includes proteins that do not fall into the major defined categories but are nonetheless functionally linked to the drought stress response and associated recovery mechanisms.

Roles of osmoprotectant synthesis, cell wall reinforcement, and cytoskeleton remodeling in quinoa's response to drought

Drought stress leads to decreased cellular water potential, resulting in plant tissue dehydration and directly affecting the flexibility and extensibility of the cell wall⁵³. This latter, a crucial subcellular structure, plays a key role in modulating drought stress signaling and serves as an important target for stress-induced changes in protein abundance. In addition, plants have evolved several strategies to regulate water content through various cellular processes, including cell wall modifications and remodeling that help minimize water loss and prevent dehydration^{54,55}. In the present study, proteins related to the cell defense pathway and cytoskeleton organization were identified and could potentially contribute to cellular protection against drought-induced damage (Table 2). Among them, we highlight proteins involved in cell wall metabolism, such as S-adenosylmethionine synthetase (SAMS) and caffeoyl-CoA O-methyltransferase (CCoAOMT), two key enzymes in lignin biosynthesis, which play a vital role in enhancing plant resistance to abiotic stress⁵⁶. S-adenosylmethionine synthetase was increased during short-term drought stress (D1W) but returned to control levels under prolonged stress conditions (D2W), suggesting its role in maintaining cellular homeostasis by enhancing methionine and osmoregulant metabolism, as previously reported by Wang et al.⁵⁷. Lignin content has been widely reported as an effective indicator of drought tolerance. Since CCoAOMT is a key enzyme in lignin biosynthesis and is generally upregulated under drought stress⁵⁸, its decreased abundance during prolonged drought, followed by recovery upon rehydration, with plants displaying a normal phenotype compared to the control, suggests that CCoAOMT may play a transient or adaptive role in the drought response. Our results are consistent with a previous study showing upregulation of CCoAOMT in the halophyte *Eutrema salsugineum* under salt stress⁵⁹, as well as in *Medicago sativa*⁶⁰ subjected to drought stress. Collectively, these studies indicate that CCoAOMT functions as a positive regulator of lignin biosynthesis and enhances stress tolerance. This effect appears to be mediated through modulation of stress-responsive pathways involved in reactive oxygen species (ROS) scavenging and oxidized protein detoxification, as well as through the regulation of lignin and soluble sugar biosynthesis. As an increased abundance of CCoAOMT has not been reported in major halophyte species according to the available literature, our findings suggest that CCoAOMT is a key regulatory gene in the lignin biosynthesis pathway and may represent a novel potential marker protein for drought stress response. (Fig. 4a).

			Location ^b	Peptides (95%)	Unused	Total	Sequence coverage (95%)	Treatment effect ^c			
N°	Accession ^a #	Name						D1W	D2W	R1W	R2W
Cell wall reinforcement and cytoskeleton remodeling											
1	sp Q43095 CAMT_POPTM	Caffeoyl-CoA O-methyltransferase	Cyt.	2	4	4	18,2				
2	sp Q7XZR1 METK1_ATRNU	S-adenosylmethionine synthase 1	Cyt.	3	5,63	5,72	13,1				
3	sp P30165 ACT2_PEA	Actin-2	Cytosk.	6	10,17	10,24	18,9				
4	sp Q9XF42 PROF3_MALDO	Profilin-3	Cytosk.	4	4	4	17,6				
5	sp Q9ZRA4 AB19A_PRUPE	Auxin- ABP19A	Apoplast	16	4,83	4,87	15,3				
6	sp Q56YA5 SGAT_ARATH	Serine-aminotransferase-AGT1	Perox.	5	8,01	8,01	16,5				
7	sp Q9SZJ5 GLYM1_ARATH	Serine hydroxymethyltransferase 1	Mit.	13	18,73	18,74	25,3				
8	sp P38559 GLNA1_MAIZE	Glutamine synthetase isozyme 1	Cyt.	4	2	6,42	16,5				
9	sp P04353 CALM_SPIOL	Calmodulin	Cyt.	7	7,58	7,67	55,7				
10	sp F4I4B6 EX84A_ARATH	Exocyst complex EXO84A	Cyt.	1	2	2	1,3				
ROS detoxification and Heat Shock Proteins											
11	sp P93258 SODC1_MESCR	SOD (Cu-Zn)	Cyt.	1	2	2	8,6				
12	sp Q01297 CATA1_RICCO	Catalase isozyme 1	Perox.	4	2	6.1	11,2				
13	sp P49317 CATA3_NICPL	Catalase isozyme 3	Perox.	5	6.1	6.2	13				
14	sp A9PCL4 PRX2_POPTR	Peroxioredoxin	Cyt.	2	4	4	10.5				
15	sp Q10N21 APX1_ORYSJ	L-ascorbate peroxidase 1	Cyt.	4	5,62	5,67	13,2				
16	sp Q9FE01 APX2_ORYSJ	L-ascorbate peroxidase 2	Cyt.	4	2,01	4,01	16,7				
17	sp Q9LFA3 MDAR1_ARATH	Monodehydroascorbate reductase 1	Perox.	1	2,13	2,14	3				
18	sp Q6NPF9 TRXY1_ARATH	Thioredoxin Y1	Chl.	1	2	2,06	6,4				
19	sp Q9SGS4 CDSP_ARATH	Thioredoxin-like protein CDSP32	Chl.	1	2,12	2,13	3				
20	sp O65398 GLX1_ARATH	Lactoylglutathione lyase GLX1	Cyt.	2	2	2,09	7,8				
21	sp F4JFN3 HS906_ARATH	HSP90-6	Mit.	1	1,3	1,49	2				
22	sp P55737 HS902_ARATH	HSP90-2	Cyt.	6	5,72	11,06	12,2				
23	sp P27323 HS901_ARATH	HSP90-1	Cyt.	6	11,29	11,36	13,3				
24	sp O65719 HSP7C_ARATH	HSP70-3	Cyt.	20	1,38	34,99	38,2				
25	sp P29357 HSP7E_SPIOL	HSP70-E	Chl.	26	45,68	45,68	50,5				
26	sp Q9FVT2 EF1G2_ARATH	Probable elongation fact. 1-gamma 2	Cyt.	3	2,86	4,59	8,2				
27	sp P42895 ENO2_MAIZE	Enolase 2	Cyt.	7	11,83	11,87	21,3				
28	sp Q9SRZ6 ICDHC_ARATH	Isocitrate dehydrogenase [NADP]	Cyt.	5	8,26	8,28	16,6				
29	sp P52577 IFRH_ARATH	Isoflavone reductase homolog	Cyt.	1	2,1	2,11	3,9				
Energy and carbon metabolism											
30	sp P0DKC4 PGP1B_ARATH	Phosphoglycolate phosphatase 1B	Chl.	4	7,05	7,09	9,7				
31	sp Q43299 CAPP_AMAHP	Phosphoenolpruvate carboxylase	Cyt.	7	7,74	15,14	9,8				
32	sp P30792 PMGI_MAIZE	2,3-bisphosphoglycerate mutase	Cyt.	4	6,03	6,03	8,1				
33	sp P19595 UGPA_SOLTU	UTP-glucose-1-phosphate uridylyltransferase	Cyt.	7	10,33	10,48	13				
34	sp Q42806 KPYC_SOYBN	Pyruvate kinase, cytosolic isozyme	Cyt.	5	10,15	10,16	15,7				
35	sp Q40677 ALFP_ORYSJ	Fructose-bisphosphate aldolase	Chl.	27	10,38	22,8	43,6				
36	sp Q5N725 ALFC3_ORYSJ	Fructose-bisphosphate aldolase 3	Cyt.	10	4,03	10,05	28,2				
37	sp P15719 MDHP_MAIZE	Malate dehydrogenase [NADP]	Chl.	2	3,2	3,38	4,9				
38	sp P12783 PGKY_WHEAT	Phosphoglycerate kinase	Cyt.	5	2	8,31	13,7				
39	sp Q42961 PGKH_TOBAC	Phosphoglycerate kinase	Chl.	15	6,95	23,43	28,1				
40	sp P19866 G3PA_SPIOL	GAPDH A	Cyt.	14	2,21	21,08	32,7				
41	sp P12860 G3PB_SPIOL	GAPDH B	Cyt.	10	12	20,03	26,4				
42	sp O20250 TKTC_SPIOL	Transketolase	Chl.	28	38,58	38,52	43,1				
43	sp P48496 TPIC_SPIOL	Triosephosphate isomerase	Chl.	12	17,25	17,23	42,6				
44	sp P46285 S17P_WHEAT	Sedoheptulose-1,7-bisphosphatase	Chl.	7	2	8,14	12,7				
45	sp Q42796 F16P1_SOYBN	Fructose-1,6-bisphosphatase	Chl.	4	2	8	13,4				
46	sp P55232 GLGS_BETVU	AGPS1	Chl.	3	7,22	7,28	7				
47	sp Q9C524 SCRK6_ARATH	Fructokinase-6	Chl.	1	2	2	3,9				
48	sp Q9SE60 MTHR1_ARATH	Methylenetetrahydrofolate reductase 1	Cyt.	5	7,43	7,46	7,4				
49	sp Q9LH76 RHM3_ARATH	UDP-glucose 4,6-dehydratase	Cyt.	1	2	2,02	3,5				
50	sp P81406 GAPN_PEA	NADP-dependent GAPDH	Cyt.	6	4,01	6,71	17,7				
51	sp Q6Z0I4 FABI1_ORYSJ	Enoyl-ACP reductase [NADH]	Cyt.	4	5,21	5,24	15,5				
52	sp Q2QLY4 MTE2_ORYSJ	5-methyltetrahydropteroyltriglutamate	Cyt.	26	12,16	29,59	26,4				
53	sp P49608 ACOC_CUCMA	Aconitate hydratase	Cyt.	6	5,9	6,26	10,1				
54	sp P16016 CAHC_SPIOL	Carbonic anhydrase	Chl.	23	20,23	20,24	38,6				
55	sp P27140 BCA1_ARATH	Beta carbonic anhydrase 1	Chl.	9	8,14	12,35	17,6				
56	sp P00825 ATPB_SPIOL	ATP synthase subunit beta	Chl.	43	8	51,46	67,5				
57	sp P06450 ATPA_SPIOL	ATP synthase subunit alpha	Chl.	16	27,08	27,2	28,4				
58	sp Q9MRR1 ATPB_CINCA	ATP synthase subunit beta	Chl.	45	53,29	53,29	71,7				
59	sp Q49KZ1 ATPB_EUCGG	ATP synthase subunit beta	Chl.	37	2,28	44,84	69,9				
60	sp P29685 ATPBM_HEVBR	ATP synthase subunit beta	Mit.	16	22,58	24,63	33,8				
61	sp Q39291 VATA_BRANA	V-typoe proton ATPase catalytic subunit A	Vac.	2	5,14	5,22	5,8				

Table 2. Leaf proteome variations among treatments using the SWATH-MS analysis, by comparing control vs Drought stress for one week (D1W) or two weeks (D2W), and control vs recovery treatments (R1W) and (R2W). Proteins with fold changes ≥ 1.5 or ≤ 0.66 and p -values ≤ 0.05 were considered were considered significantly different in abundance. The different colors represented different levels of $\log_2$ (fold change). Red indicates a decrease in protein abundance, and blue indicates an increase. The darker colors represent the greater fold change

^aAccession number is assigned from UniProtKB database.
^bProtein Subcellular Location: Chl., Chloroplast; Cyt., Cytoplasm; Cytosk., Cytoskeleton; Mit., Mitochondrion; Nuc., Nucleus; RE., Endoplasmic reticulum; Vac., Vacuole; Perox., Peroxisome. ^cVisualization of protein fold changes in quinoa leaves under drought stress and recovery treatments. Red indicates a decrease, blue indicates an increase, and darker shades represent greater fold-change magnitudes.

Photosynthesis									
62	sp P12359 PSBO_SPIOL	Oxygen-evolving enhancer protein 1	Chl.	17	23,28	23,22	36,8		
63	sp P23322 PSBO_SOLL	Oxygen-evolving enhancer protein 1	Chl.	16	4	17,9	36,8		
64	sp O49344 PSBP2_ARATH	Oxygen-evolving enhancer protein 2-2	Chl.	6	2	2,3	27,2		
65	sp Q42840 HEM6_HORVU	Oxygen-dependent coproporphyrinogen-oxidase	Chl.	6	6,1	6,11	8,2		
66	sp O82660 P2SAF_ARATH	PSII stability/assembly factor HCF136	Chl.	1	2,01	2,03	2,5		
67	sp P00071 CYC_PASSA	Cytochrome c	Mit.	5	6,04	6,05	33,3		
68	sp P05314 NIR_SPIOL	Ferredoxin--nitrite reductase	Chl.	7	12,51	12,53	15,3		
69	sp Q9ZNZ7 GLT11_ARATH	Ferredoxin-dependent glutamate synthase	Chl.	16	5,72	28,9	13		
70	sp Q40460 RCA1_TOBAC	RuBisCO activase 1	Chl.	35	26,08	26,17	36,4		
71	sp P10647 RBSO_SOLTU	RuBisCO small subunit C	Chl.	14	11,03	11,07	27,6		
72	sp O65349 RBS_CAPAN	RuBisCO small subunit	Chl.	13	4	10,2	25,1		
73	sp P04714 RBS1_PETHY	RuBisCO small subunit SSU8	Chl.	13	2,4	6,73	21,1		
74	sp P04715 RBS2_PETHY	RuBisCO small subunit SSU11A	Chl.	12	2,52	6,87	21,1		
75	sp Q05798 RBL_CERDE	RuBisCO large subunit	Chl.	74	4,55	54,72	57,9		
76	sp P51994 RBL_BAMML	RuBisCO large subunit	Chl.	51	14,52	45,69	44,6		
77	sp Q9MUT6 RBL_MESVI	RuBisCO large subunit	Chl.	59	6	45,68	34,1		
78	sp P19160 RBL_ATRPA	RuBisCO large subunit	Chl.	94	85,18	85,18	72,9		
79	sp P16306 RBL_AMAHP	RuBisCO large subunit	Chl.	81	3,51	70,13	53,3		
80	sp P34794 RUB2_BRANA	60 kDa chaperonin subunit alpha	Chl.	14	22,02	22,05	20,6		
81	sp P08926 RUBA_PEA	60 kDa chaperonin subunit alpha	Chl.	13	2	21,17	19,9		
82	sp P08927 RUBB_PEA	60 kDa chaperonin subunit beta	Chl.	12	21,15	21,26	27,9		
DNA, RNA assembly and structure									
83	sp F6H9A9 THI41_VITVI	Thiamine thiazole synthase 1	Chl.	3	2	5,39	12,8		
84	sp A9SME1 THI42_PHYPA	Thiamine thiazole synthase 2	Chl.	6	2	5,39	16,3		
85	sp Q9C944 H2AV3_ARATH	Probable histone H2A variant 3	Nuc.	4	3,83	3,93	22,4		
86	sp Q9LGH8 H2B8_ORYSJ	Histone H2B.8, Q76H85	Nuc.	3	6,43	6,44	22,9		
87	sp Q76H85 H4_SILLA	Histone H4	Nuc.	1	1,52	1,71	11,7		
88	sp Q05966 GRP10_BRANA	Glycine-rich RNA-binding protein 10	Cyt.	3	2,08	2,09	16		
89	sp Q6ZK00 14333_ORYSJ	14-3-3-like protein GF14-C	Cyt.	7	4,01	13,97	30,1		
90	sp A6BM50 RPOB_GNEPA	DNA-directed RNA polymerase subunit β	Chl.	1	2	2	1,2		
91	sp Q9ZUU4 CP29B_ARATH	RNA-binding protein CP29B	Chl.	1	2,06	2,07	3,1		
92	sp Q8W034 RNP1_ARATH	Heterogeneous nuclear ribonucleoprotein	Nuc.	1	2	2	3,7		
Amino acid and Protein biosynthesis									
93	sp P34811 EFGC1_SOYBN	Elongation factor G-1,	Chl.	9	11,8	11,85	18,3		
94	sp I1K0K6 EFGC2_SOYBN	Elongation factor G-2,	Chl.	9	2	11,85	18,5		
95	sp P82412 RRP3_SPIOL	30S ribosomal protein 3	Chl.	1	2,68	2,77	14		
96	sp O22518 RSSA_SOYBN	40S ribosomal protein SA	Cyt.	3	6,04	6,04	11,6		
97	sp Q9STY6 RS202_ARATH	40S ribosomal protein S20-2	Cyt.	2	2	2	19,7		
98	sp P82180 RK9_SPIOL	50S ribosomal protein L9	Chl.	1	2	2,02	8,2		
99	sp Q9MAP3 RK11_ARATH	50S ribosomal protein L11	Chl.	1	2,02	2,02	6,3		
100	sp Q9FZ47 ACR11_ARATH	ACT domain-containing protein ACR11	Chl.	1	2	2	2,8		
101	sp O04895 BADH_AMAHP	Betaine aldehyde dehydrogenase	Chl.	3	2	4,02	7,2		
102	sp O49954 GCSP_SOLTU	Glycine dehydrogenase	Mit.	11	2,17	15,8	18,3		
103	sp Q9FEA2 SYEM_ARATH	Glutamate-tRNA ligase	Chl./mit.	1	1,51	1,68	1,8		
104	sp Q9SRV5 METE2_ARATH	5-methyltetrahydropteroyltriglutamate-	Cyt.	15	3,75	19,45	22,1		
105	sp O23735 CYK2_BRAJU	Cysteine synthase	Cyt.	2	1,57	3,89	7,4		
106	sp Q94JQ4 RIDA_ARATH	Reactive Intermediate Deaminase A	Chl.	1	2,3	2,33	7		
107	sp P54847 ACCO3_CUCME	1-aminocyclopropane-1-carboxylate oxidase 3	Cyt.	1	2	2	5,3		
108	sp Q65XK0 ILV5_ORYSJ	Ketol-acid reductoisomerase (KARI)	Chl.	11	8,11	12,63	17,3		
109	sp P93395 PSB6_TOBAC	Proteasome subunit beta type-6	Nuc.	1	1,76	1,98	6,4		
Miscellaneous									
110	sp Q9LYA9 CP41A_ARATH	Stem-loop binding protein of 41 kDa a	Chl.	4	5,29	5,36	17,7		
111	sp Q9SA52 CP41B_ARATH	Stem-loop binding protein of 41 kDa b	Chl.	3	4,61	4,63	9,3		
112	sp A8DMN5 FL1_MAIZE	Protein FLOURY 1	RE	1	1,57	1,74	4		
113	sp Q05968 PR1_HORVU	Pathogenesis-related protein 1	Cyt.	3	4	4	21,3		
114	sp Q9SCN8 CD48D_ARATH	Cell division control protein 48 homolog	Cyt.	5	5,58	5,65	6,3		

Table 2 (continued)

Additional proteins associated with cytoskeleton rearrangements were also identified in the present study, among them, actin and profilin, were detected in the quinoa leaf proteome under drought conditions. In previous proteomic studies, these cytoskeleton-related proteins were found to decrease in response to drought stress in a susceptible cultivar of *Hordeum vulgare*, but increased in a drought-tolerant cultivar, suggesting their involvement in plasma membrane reinforcement and enhancement of cell wall strength⁶¹. Our study showed a significant increase in actin levels under drought stress, with a pronounced effect observed during the short-term period of one week (D1W), and an attenuated response following the recovery period. In addition, profilin, a protein that binds to actin and controlling the growth of actin microfilaments, is decreased by drought (D1W) and return to control levels where stress was prolonged for two weeks. This negative correlation between actin and profilin under drought stress may suggest a role in modulating cytoskeleton organization during the cell division cycle, as previously discussed by Wu et al.⁶². This modulation may help explain the rapid rearrangements of cytoskeletal and chloroplast structures in quinoa during drought recovery regimes, as reported in previous work¹¹.

A protein called glutamine synthetase (GS), which may contribute to the osmotic adjustment under drought stress by protecting enzymes and membranes, was identified in our study. Glutamine synthetase is known to be involved in amino acid biosynthesis and plays a pivotal role in nitrogen assimilation by reassimilating NH_4^+ released from photorespiration and proteolysis in plants subjected to water deficit, through the synthesis of glutamine and precursors of proline⁶³. Its abundance was significantly decreased, particularly following

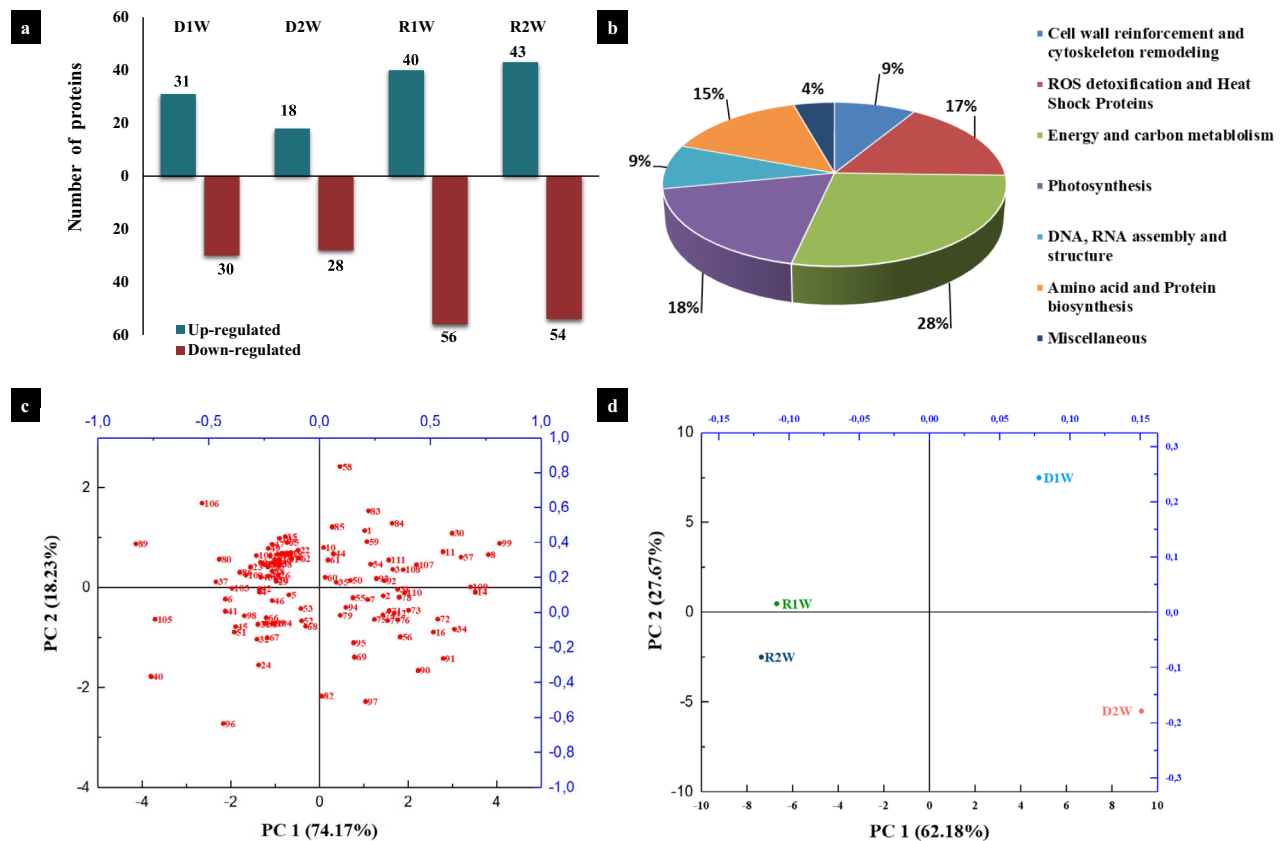


Fig. 3. (a) The number of leaf proteins differing significantly in abundance in drought stressed (D1W and D2W) and rewatered quinoa plants (R1W and R2W) compared with well-watered controls, based on data in Table 2. (b) Functional category distribution of the 114 identified proteins. (c) Spatial distribution of differentially expressed proteins based on principal component analysis (PCA), using fold-change values relative to the control. (d) PCA score plot derived from fold-change values of the differentially expressed proteins under different treatments (D1W, D2W, R1W, and R2W).

prolonged drought stress (D2W), and subsequently recovered to control levels after the rewatering phase. Such a pattern of variations suggests that prolonged drought stress induced a disruption of cellular homeostasis, which was more rapidly restored during the recovery phase.

The complex EXO84A protein was identified and is suggested to play a key role in cell wall defense pathways and in the transport of primary and secondary metabolites⁶⁴. However, no data have been reported on its abundance variation under drought or salinity stress in either glycophyte or halophyte species. In the present study, EXO84A abundance was high during the first week of drought stress and decreased slightly following stress removal. This pattern may reflect an adaptive mechanism contributing to drought tolerance and could serve as a potential marker for early stress response and recovery dynamics.

ROS detoxification and Heat Shock Proteins as mechanisms associated with quinoa's response during drought stress and recovery

Water deficit is widely recognized as a major inducer of oxidative stress in plants⁶⁵. Drought stress impairs photosynthesis and disrupts normal cellular metabolism, resulting in excessive production of ROS, which can cause severe oxidative damage to cellular components, including membrane lipids, proteins, and nucleic acids⁶⁶. To mitigate this damage, plants have evolved a well-organized system of enzymatic and non-enzymatic antioxidant defenses that regulate ROS homeostasis and protect cells from oxidative stress⁶⁷. Enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione peroxidase (GPX) play central roles in scavenging ROS, often functioning in a sequential and synergistic manner⁶⁵. Other enzymes including thioredoxin play a key role in the plant's defense system against oxidative damage by reducing the disulfide bonds of oxidized proteins⁶⁸. These proteins were detected in our comparative proteome study (Table 2), showing significant changes under different drought stress conditions, and are considered crucial for preventing oxidative damage and enhancing quinoa's drought tolerance. Notably, a slight increase of SOD, two CAT isoforms, and two APX isoforms was observed specifically during the prolonged drought treatment (D2W), with this increase becoming more pronounced after the two-week recovery period, particularly for the two CAT isoforms. The enhanced activity of these antioxidant enzymes was also reported in *Musa acuminata* leaves after 14 days of drought stress⁶⁹. In contrast, SOD, APX, and lactoylglutathione showed a decrease in

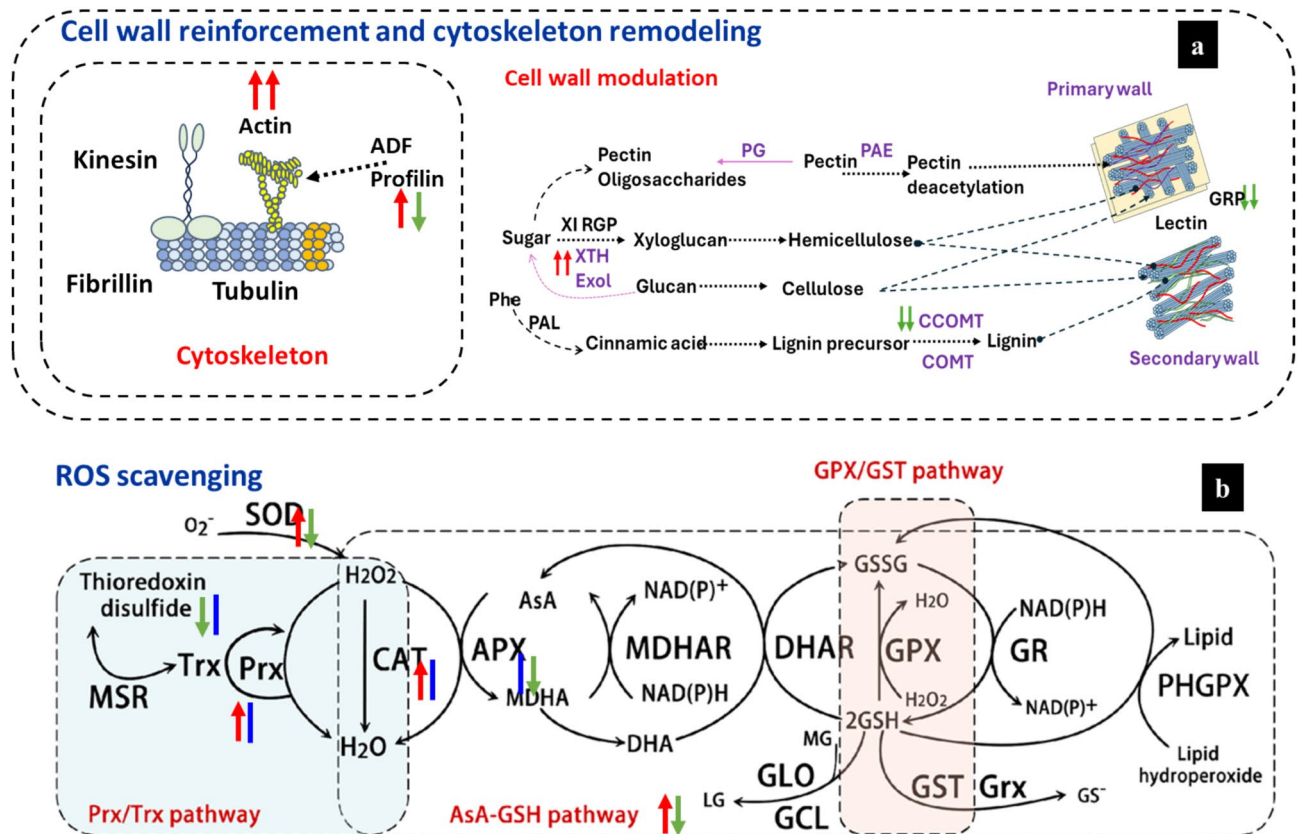


Fig. 4. Schematic representation of proteins involved in (a) cell wall reinforcement and cytoskeleton remodeling, and (b) ROS scavenging under drought conditions (modified from⁵⁷). The differentially accumulated proteins are indicated by colored symbols: (↑↓). Red up arrow, upregulated; green down arrow; blue vertical line, protein abundance remained at control level. Proteins showing a significant difference between drought treatments and control are marked with two symbols: the left symbol represents the change in abundance after drought treatment (D2W), and the right symbol represents the abundance after the recovery period (R2W). Abbreviation: ADF: actin depolymerizing factor; ALDH: aldehyde dehydrogenase; AsA: ascorbate; APX: ascorbate peroxidase; Exol: glucan exohydrolase; COMT: caffeic acid 3-O-methyltransferase; CCOMT: caffeoyl-CoA O-methyl-transferase; CAT: catalase; DHAR: dehydroascorbate reductase; DHA: dehydroascorbate; GME: GDP-d-mannose-3',5'-epimerase; GCL: glutamate-cysteine ligase; Grx: glutaredoxin; GPX: glutathione peroxidase; GR: glutathione reductase; GST: glutathione S-transferase; GSSG: glutathione disulfide; GSH: glutathione; GRP: glycine-rich protein; GLO: glyoxalase; LG: (R)-S-lactoylglutathione; MSR: methionine sulfoxide reductase; MG: methylglyoxal; MDHAR: monodehydroascorbate reductase; MDHA: monodehydroascorbate; PAE: pectin acetyltransferase; PAL: phenylalanine ammonia-lyase; PG: pectin depolymerase; PHGPX: phospholipid hydroperoxide glutathione peroxidase; POD: peroxidase; Prx: peroxiredoxin; RGP: reversibly glycosylated polypeptide; SOD: superoxide dismutase; Trx: thioredoxin; XI: xylanase inhibitor; XTH: xyloglucan endotransglycosylase.

abundance only after the rehydration phase. This decrease upon rewetting, along with their low abundance during drought stress (D1W and D2W) compared to the control, may be associated with reduced oxidative damage and could reflect an adjustment in defense strategy during the post-drought recovery phase. Similar results were reported by Sofo et al.⁷⁰ in *Prunus amygdalus* and by Furlan et al.⁷¹ in *Arachis hypogaea*, where the activities of antioxidant enzymes: APX, monodehydroascorbate reductase (MR), dehydroascorbate reductase (DR), and glutathione reductase (GR) were decreased during the rewetting phase, with levels lower than those observed in drought-stressed plants. These findings suggest that ROS signaling may trigger negative feedback loops to limit enzyme expression as part of a broader stress adaptation strategy, shifting resources toward other defense mechanisms, such as osmoprotectant synthesis or cell wall reinforcement, as discussed above in our study (Fig. 4b).

To cope with environmental stress, plants have developed complex defense mechanisms, including molecular responses such as altered gene expression and the synthesis of stress-responsive proteins⁷². Among these, heat shock proteins (HSPs) play a vital role in enabling plants to withstand and adapt to external stimuli⁷³. As previously documented, drought stress leads to an increased abundance of various HSPs^{74,75}. In line with this, our study revealed a significant upregulation of three isoforms of HSP90, which showed high abundance during prolonged stress and decreased levels following the recovery period. However, a decrease of two isoforms of HSP70 were detected with a high abundance after prolonged treatment (D2W). The observed abundance patterns of heat

shock proteins suggest a dynamic, stress-phase-specific regulation of these molecular chaperones. Based on the previous study⁷⁴, elevated HSP levels may contribute to the mitigation of denatured protein aggregation and the facilitation of protein refolding, processes that could support cellular homeostasis during and after drought stress. Such potential protective and regulatory roles of HSPs may be particularly relevant during the rehydration phase, when cells may experience increased protein misfolding due to osmotic fluctuations.

Photosynthetic protein dynamics supporting highly photosynthetic performance in Quinoa under drought and recovery

A total of ten differentially accumulated proteins related to RuBisCO, including five large subunits, four small subunits, and one RuBisCO activase, were identified, representing most of the photosynthesis-related proteins detected in our study. It has been reported that RuBisCO subunits are susceptible to drought stress, and their induction or degradation under such conditions has shown contradictory results⁷⁶. In general, the large and small subunits of RuBisCO tend to decrease under drought stress and increase following re-watering. However, in our study, seven RuBisCO subunits isoforms (both large and small) were increased during drought stress (D1W and D2W) and continued to increase during the subsequent recovery period, with particularly high abundance observed after rehydration. Additionally, three other isoforms, RuBisCO activase 1 and two RuBisCO small subunits, showed a slight decrease in abundance during drought stress but were increased following the recovery phase.

Since RuBisCO and its subunits are key photosynthetic enzyme, directly linked to photosynthetic metabolism and plays a crucial role in maintaining carbon, nitrogen balance in plants⁷⁷, their abundance profile change in our study suggest that the photosynthetic process was only mildly affected by drought stress. Even more, our results indicate a clear enhancement and rapid recovery of the RuBisCO activity once the drought stress was removed. To align with previous reports, there is a wide discrepancy in the literature regarding changes in Rubisco levels under stress conditions and the effects of drought on RuBisCO synthesis, content, and stability remain insufficiently understood. Demirevska et al.⁷⁸ demonstrated that the decrease of Rubisco level in *Triticum aestivum* was attributed to oxidative damage and possible protein degradation in response to drought stress. The rapid recovery of photosynthetic proteins (such as Rubisco activase) following rewetting along with elevated levels of antioxidant proteins, has also been reported in *Cistus albidus*⁷⁹ and in *Carthamus tinctorius*⁷⁶.

Chloroplast ferredoxin has been demonstrated to be involved in numerous essential metabolic processes and is recognized primarily as a component of the photosynthetic electron transport chain^{80,81}. Two ferredoxin-related proteins involved in photorespiration and primary nitrogen assimilation have been identified: Ferredoxin-nitrite reductase (Fd-NiR, pathway of nitrate assimilation) and Ferredoxin-dependent glutamate synthase (Fd-GOGAT; reassimilation of ammonium). The abundance levels of these proteins showed a decrease under drought stress, with the most pronounced reduction occurring during the prolonged drought period (D2W), followed by significant recovery during the subsequent rewetting phase. Similar results have been reported in leaves of *Triticum turgidum*^{82,83} and *Carthamus tinctorius*⁷⁶, where drought stress induced a decrease in the abundance of Ferredoxin-NADP reductase, Ferredoxin-nitrite reductase, and Ferredoxin-glutamate dehydrogenase. Taken together, the low levels of Fd-NiR and Fd-GOGAT under drought stress, and their restoration to control values following rehydration, may indicate a downregulation of photo-dependent nitrogen assimilation. This pattern could reflect a physiological adjustment to limit energy- and electron-consuming processes when photosynthetic activity is reduced (Fig. 5). Such changes may help limit the potential for ROS accumulation and contribute to the maintenance of photosynthetic performance. This metabolic shift could facilitate a rapid recovery of nitrogen assimilation once water availability is restored.

Interestingly, drought-induced changes in the abundance levels of Oxygen-Evolving Enhancer proteins (OEE1 and OEE2), which play supportive and stabilizing roles in the water-splitting reaction, were observed in our study. Their levels increased under drought stress, with the most prominent effect occurring during the first week (D1W), followed by a slight decrease after the rewetting period. The increased abundance of OEE1 and OEE2, components of the OEC complex in Photosystem II (PSII), suggests a protective mechanism in which oxygen production is maintained to enhance the stability of PSII under severe drought conditions. This response likely reflects a protective and preparatory strategy aimed at preventing structural damage to PSII, stabilizing the OEC under fluctuating water availability, and facilitating rapid recovery upon rehydration (Fig. 6). These findings confirm our previous observations, which demonstrated that quinoa maintains robust photosynthetic machinery under both drought and salinity stress^{10,11}, as evidenced by the high stability of PSI and PSII functions, and the preservation of functional chloroplast ultrastructure. Our previous studies demonstrated that quinoa maintains active electron transport chains across different drought regimes and possesses a strong capacity to repair damaged PSII protein complexes following stress.

Proteomic evidence of energy metabolism alterations in quinoa during drought and rewetting

Plant growth and development under drought stress require substantial amounts of energy, which is primarily generated through carbohydrate metabolism pathways (Fig. 6) such as glycolysis and the tricarboxylic acid (TCA) cycle⁸⁴. Consistent with this, the majority of proteins identified in the present study were involved in carbohydrate and energy metabolism (Table 2). Drought induced a decreased abundance of several key enzymes, including 2,3-bisphosphoglycerate mutase, UTP-glucose-1-phosphate uridylyltransferase, pyruvate kinase, fructose-bisphosphate aldolase, and malate dehydrogenase, starting from the first week of water deficit and continuing into the subsequent recovery periods. The smallest reduction was observed during prolonged period of drought (D2W). These enzymes play a crucial role in the primary pathways of energy production in higher plants, as they are key components of both glycolysis and the tricarboxylic acid (TCA) cycle, and have been reported to contribute to energy supply under abiotic stress conditions⁸⁵. The observed decrease in the

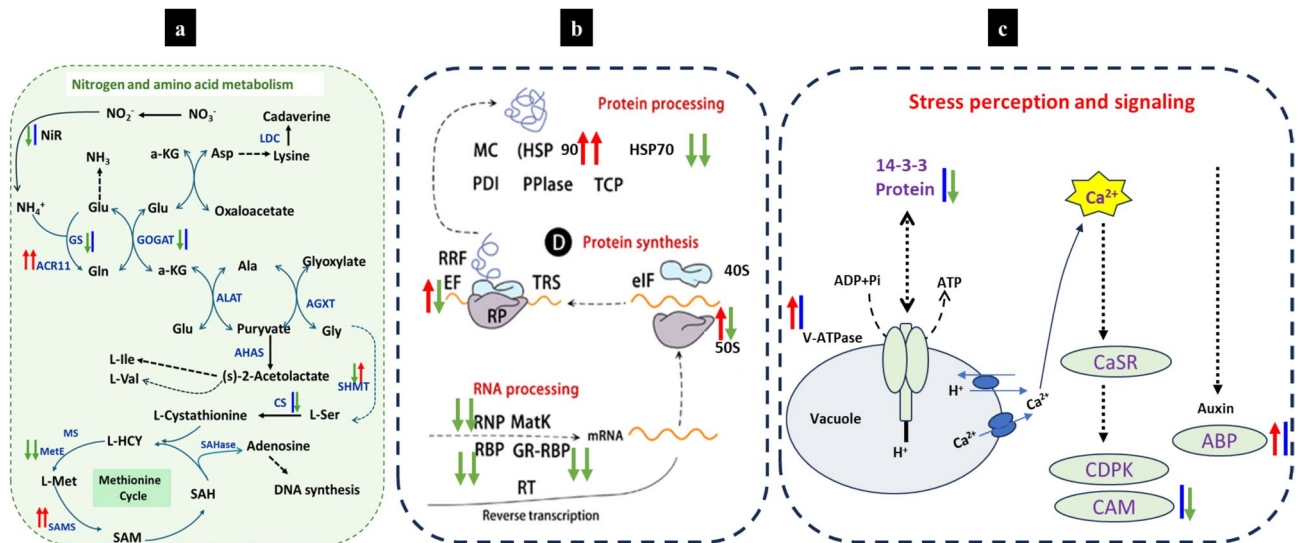


Fig. 5. A putative synergistic responsive network of drought responsive proteins involved in (a) Nitrogen and amino acid metabolism, (b) Protein synthesis, degradation, RNA processing and (c) Stress perception and signaling (modified from⁵⁷). The differentially accumulated proteins are indicated by colored symbols: (↑↓). Red up arrow, upregulated; green down arrow; blue vertical line, protein abundance remained at control level. Proteins showing a significant difference between drought treatments and control are marked with two symbols: the left symbol represents the change in abundance after drought treatment (D2W), and the right symbol represents the abundance after the recovery period (R2W). Abbreviation: 14-3-3: 14-3-3 protein; ABP: auxin-binding protein; AHAS: acetohydroxyacid synthase; ALAT: alanine aminotransferase; AGXT: alanine-glyoxylate aminotransferase; AST: aspartate aminotransferase; CaSR: calcium sensing receptor; CDPK: calcium-dependent protein kinase; CAM: calmodulin; CS: cysteine synthase; EF: elongation factor; GOGAT: glutamate synthase; GS: glutamine synthetase; GR-RBP: glycine-rich RNA binding protein; HSP: heat shock protein; MetE: 5-methyltetrahydropteroyltryglutamate homocysteine methyltransferase; MatK: maturase K; MC: molecular chaperone; NIR: nitrite reductase; PPIase: peptidyl-prolyl *cis-trans* isomerase; PDI: protein disulphide isomerase; RT: retrotransposon protein; RNP: ribonucleoprotein; RP: ribosomal protein; RBP: RNA binding protein; SAHase: adenosyl homocysteinase; SAH: S-adenosyl-L-homocysteine; SAM: S-adenosylmethionine; SAMS: S-adenosylmethionine synthase; SHMT: serine transhydroxymethyl transferase; TCP: T-complex protein; V-ATPase: vacuolar H⁺-ATPase; α-KG: α-ketoglutarate.

abundance of these proteins may be associated with decreased ATP production, which appears to be restored following rewatering, reflecting quinoa's capacity for metabolic recovery under drought stress.

A high abundance of phosphoglycerate kinase (PGK), a key enzyme in central energy production pathways, was observed with upregulation in response to drought stress and recovery periods. Similar increased abundance of PGK has been observed in *Vitis vinifera*⁸⁶ and *Eucalyptus saligna*⁸⁷, resulting in increased ATP production in cells and suggesting a potential role for PGK in enhancing plant tolerance to drought stress.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a well-known target for oxidative modification under stress conditions, showed an increase of both identified isoforms (GAPDH-A and GAPDH-B) under drought stress in our study. Similar findings have been reported in *Oryza sativa*⁸⁸, suggesting that GAPDH may contribute to the protection of photosystem II, with drought tolerance potentially mediated through photosynthesis-related adaptations involving NADP(H) homeostasis.

To cope with drought stress and maintain growth, plants require increased energy production, leading to a higher demand for ATP. In our study, five ATP synthase subunits were identified, all showing an increased abundance from the first week of drought treatment. Previous studies have similarly reported increased abundance of ATP synthase subunits under salt stress in quinoa⁸⁹ and drought stress in *Hordeum vulgare*⁹⁰. The notable increase in ATPase subunits observed in our study, along with elevated levels of PGK and GAPDH, indicates a high metabolic demand for NADPH and ATP as supplementary energy sources. These findings suggest that quinoa copes with drought stress by enhancing energy production, thereby protecting the plant from stress-induced damage.

Drought-induced modulation of amino acid metabolism and protein biosynthesis

Extensive amino acid accumulation, arising from stress-induced modifications in amino acid metabolism, is a well-documented plant strategy for mitigating the adverse effects of abiotic stresses such as drought⁹¹. In our study, we identified several proteins involved in the amino acid metabolism pathway, including the ACT domain-containing protein ACR11, enzymes participating in nitrogen assimilation through the regulation of glutamine synthetase (GS) levels, and ferredoxin-dependent glutamine oxoglutarate aminotransferase (Fd-GOGAT)⁹². Notably, ACR11 has been reported to influence reactive oxygen species (ROS) production in chloroplasts⁹³.

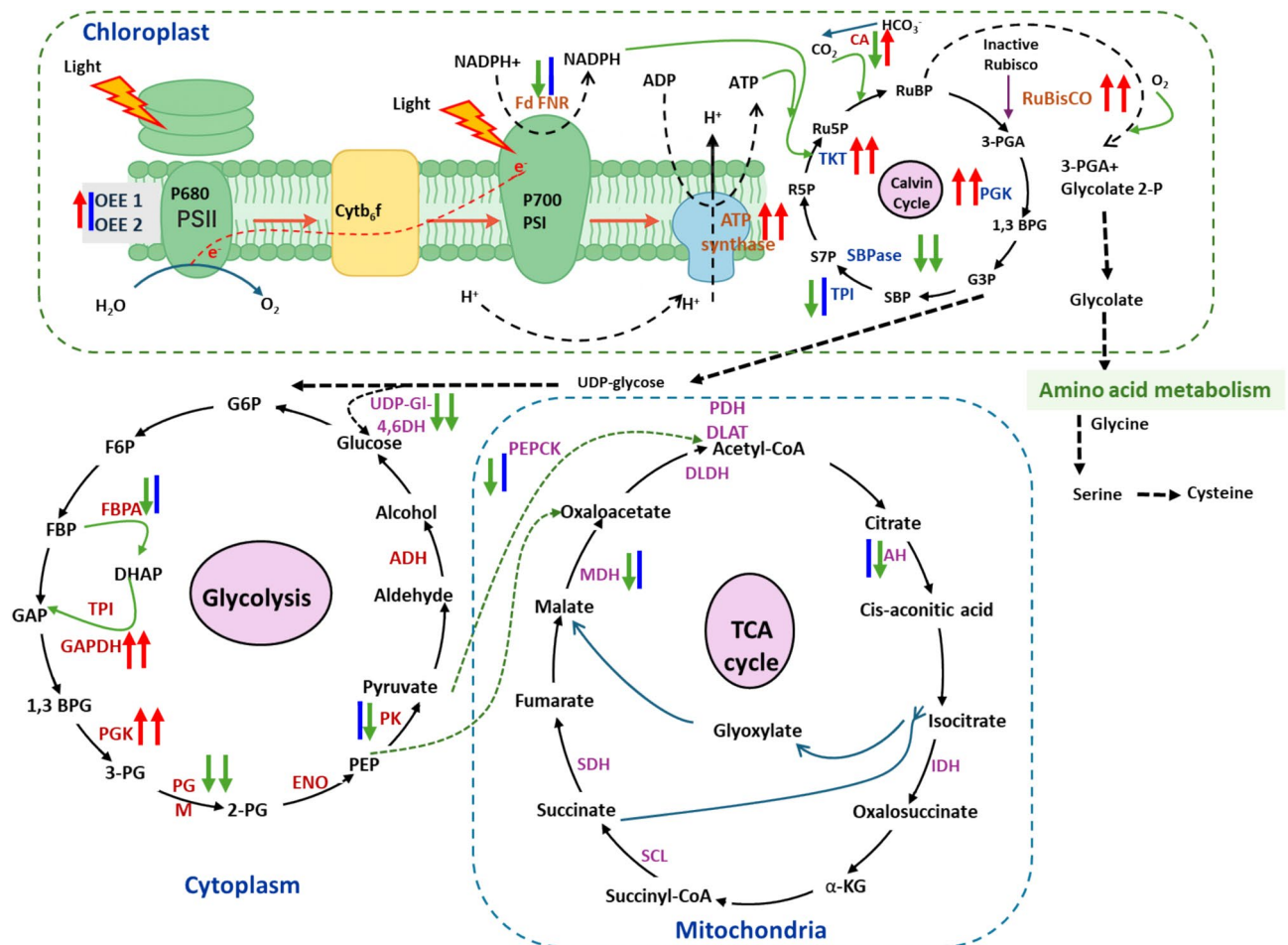


Fig. 6. Schematic representation of major drought response mechanisms in quinoa leaves by regulating diverse biological processes: Photosynthesis, Photorespiration, Calvin cycle, Glycolysis and Tricarboxylic acid (TCA) cycle. The differentially accumulated proteins are indicated by colored symbols: (↑↓). Red up arrow, upregulated; green down arrow; blue vertical line, protein abundance remained at control level. Proteins showing a significant difference between drought treatments and control are marked with two symbols: the left symbol represents the change in abundance after drought treatment (D2W), and the right symbol represents the abundance after the recovery period (R2W). Abbreviation: 1,3BPG: 1,3-bisphosphoglycerate; AH: aconitate hydratase; ADH: alcohol dehydrogenase; CA: carbonic anhydrase; FNR: chloroplast ferredoxin-NADP + oxidoreductase; Cyt b6f: cytochrome b6f. complex; DLAT: dihydrolipoamide acetyltransferase; ENO: enolase; Fd: ferredoxin; F6P: fructose 6-phosphate; FBP: fructose-1,6-bisphosphate; FBPA: fructose-bisphosphate aldolase; G6P: glucose 6-phosphate; G1P: glucose-1-phosphate; GAP: glyceraldehyde 3-phosphate; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; SBP: sedoheptulose-1,7-bisphosphatase; MDH: malate dehydrogenase; OEE oxygen-evolving enhancer proteins; PEPCK: phosphoenolpyruvate carboxykinase; PEP: phosphoenolpyruvate; PGA: phosphoglycerate; PGK: phosphoglycerate kinase; PGM: phosphoglycerate mutase; PSI: photosystem I reaction center protein; PSII: photosystem II reaction center protein; PDH: pyruvate dehydrogenase; DLDH: pyruvate dehydrogenase complex; PK: pyruvate kinase; R5P: ribose-5-phosphate; RPI: ribose-5-phosphate isomerase; Ru5P: ribulose 5-phosphate; Rubisco: ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP: ribulose-1,5-disphosphate; S7P: sedoheptulose 7-phosphate; SBPase: sedoheptulose-1,7-bisphosphatase; SDH: succinate dehydrogenase; SCL: succinyl-CoA ligase; TKT: transketolase; TPI: triosephosphate isomerase; α-KG: α-ketoglutarate.

As previously reported by Azri et al.⁹⁴, ACR11 was downregulated under drought in *Vitis sylvestris*. However, few or no studies have reported on the role of ACR11 in drought stress responses in major halophyte species. In this study, the observed increase in ACR11 during drought stress and subsequent recovery may enhance nitrogen assimilation while reducing ROS accumulation (Fig. 5). This response could reflect an adaptive mechanism contributing to drought tolerance and may serve as a potential marker of plant resilience, mediated by the maintenance of carbon and nitrogen assimilation alongside strengthened oxidative defense systems.

A protein identified called Betaine aldehyde dehydrogenase (BADH) showed a decrease under prolonged drought stress (D2W) but increased upon stress removal. This enzyme plays a pivotal role in the biosynthesis

of glycine betaine, a key osmoprotectant that accumulates in response to abiotic stresses, particularly drought and salinity, as an indicator of tolerance. Similar results have been reported in the obligate halophyte *Sesuvium portulacastrum*⁹⁵. Glycine betaine accumulation under drought conditions, may contribute to stress adaptation by stabilizing protein structures and regulating cytoplasmic osmotic potential, thereby minimizing water loss and preventing dehydration⁹⁶. Furthermore, the increase of Glutamate-tRNA ligase highlights the role of glutamate in detoxifying excess ammonia, further supporting metabolic adjustments under drought stress⁹⁷.

An important enzyme involved in the biosynthesis of hydrophobic amino acids, including valine, leucine, and isoleucine, was identified as ketol-acid reductoisomerase (KARI). In our study, KARI showed an increased abundance under drought stress, indicating that the plant actively enhances the synthesis of these hydrophobic branched-chain amino acids to stabilize proteins, maintain metabolic balance. The return to control levels after stress removal suggests that this response is stress-specific and reversible, reflecting the plant's ability to dynamically adjust amino acid metabolism according to environmental conditions. This reversible regulation also minimizes unnecessary metabolic costs once the stress is alleviated. Our findings are consistent with those of Damaris et al.⁹⁸ conducted on *Oryza sativa*, who reported an increase of KARI in salt-tolerant lines and a decrease in salt-sensitive lines under osmotic stress induced by NaCl treatment. The observed increase in hydrophobic amino acid synthesis in the present work, may further support de novo protein synthesis in a more oxidative environment, resulting in isoforms enriched with these amino acids.

Proteins associated with transcription and translation, including elongation factors (G-1 and G-2), as well as 30S, 40S, and 50S ribosomal proteins, were identified. Their abundance increased during the first week of drought stress and showed further increase during the recovery period. The induction of ribosomal proteins may enhance the translation process and ensure proper ribosome functioning under stress conditions (Fig. 5), thereby playing a key role in stress responses and cell viability, as ribosome overexpression has been shown to improve plant stress resistance⁹⁹. A previous study reported that ten ribosomal proteins increased while six decreased under drought stress in cassava (*Manihot esculenta*), demonstrating that maintaining a balance between synthesis and degradation of ribosomal proteins is essential for sustaining normal cellular metabolic activities¹⁰⁰. Furthermore, the increased abundance of elongation factors (G-1 and G-2) during the recovery period in the present study indicates differential regulation of components of the translation machinery, likely leading to enhanced protein synthesis. This response may help mitigate the effects of drought stress and facilitate the return to normal cellular conditions, as previously reported by Mohammadi et al.¹⁰¹.

Conclusion

This study was conducted to assess quinoa drought tolerance through integrative analysis of physiological and proteomic responses. While drought stress significantly reduced biomass, relative water content, and osmotic potential, plants demonstrated robust recovery upon rehydration, highlighting the quinoa's resilience. Proteomic evidence revealed several key mechanisms driving this tolerance: (i) osmoprotectant synthesis, cell wall reinforcement, and cytoskeleton remodeling, ensuring cellular stability and water balance; (ii) ROS detoxification and heat shock proteins, protecting against oxidative stress and preserving protein and membrane integrity; (iii) photosynthetic protein dynamics, with sustained RuBisCO and oxygen-evolving enhancer proteins maintaining photosynthetic efficiency and (iv) drought-driven regulation of amino acid metabolism and protein biosynthesis, supporting osmotic adjustment, nitrogen assimilation, and active translation machinery.

The dynamic regulation of these pathways during both drought and recovery phases indicates that quinoa deploys immediate protective mechanisms while also activating adaptive processes related to stress memory and post-drought metabolic reprogramming. These findings not only confirm quinoa's high resilience to water scarcity but also provide valuable insights for future applications. The identified stress-responsive proteins (such as CCoAOMT, EXO84A, ACR11, KARI, OEE1 and OEE2) may serve as potential biomarkers for breeding programs aimed at improving drought tolerance in quinoa and related crops. Moreover, the strong recovery capacity highlights quinoa's suitability as a climate-smart crop for sustainable agriculture in marginal and arid regions^{12,13,17,23,34,53,64,91,97}.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Received: 20 September 2025; Accepted: 29 April 2026

Published online: 02 June 2026

References

- Ilyas, M. et al. Drought tolerance strategies in plants: A mechanistic approach. *J. Plant Growth Regul.* **40**, 926–944. <https://doi.org/10.1007/s00344-020-10174-5> (2021).
- Ghosh, A., Kumar, A. & Biswas, G. Exponential population growth and global food security: Challenges and alternatives. In *Bioremediation of Emerging Contaminants from Soils* (eds Kumar, P. et al.) 1–20 (Elsevier, 2024). <https://doi.org/10.1016/B978-0-443-13993-2.00001-3>.
- Flowers, T. J., Galal, H. K. & Bromham, L. Evolution of halophytes: Multiple origins of salt tolerance in land plants. *Funct. Plant Biol.* **37**, 604–612. <https://doi.org/10.1071/FP09269> (2010).
- Bazile, D. et al. Worldwide evaluations of quinoa: Preliminary results from post International Year of Quinoa FAO projects in nine countries. *Front. Plant Sci.* **7**, 850. <https://doi.org/10.3389/fpls.2016.00850> (2016).
- Martinez, E. A., Fuentes, F. & Bazile, D. History of quinoa: Its origin, domestication, diversification and cultivation with particular reference to the Chilean context. In *Quinoa: Improvement and Sustainable Production* (eds Murphy, K. & Matanguihan, J.) 19–24 (Wiley-Blackwell, 2015). <https://doi.org/10.1002/9781118628041.ch2>.

6. Planella, M. T., Scherson, R. & McRostie, V. Sitio El Plomo y nuevos registros de cultígenos iniciales en cazadores del Arcaico IV en alto Maipo, Chile central. *Chungará (Arica)* **43**, 189–202. <https://doi.org/10.4067/S0717-73562011000200003> (2011).
7. Ruiz, K. B. et al. Quinoa biodiversity and sustainability for food security under climate change: A review. *Agron. Sustain. Dev.* **34**, 349–359. <https://doi.org/10.1007/s13593-013-0195-0> (2014).
8. Ruiz, K. B. et al. Quinoa, a model crop for understanding salt-tolerance mechanisms in halophytes. *Plant Biosyst.* **150**, 357–371. <https://doi.org/10.1080/11263504.2015.1027317> (2016).
9. Derbali, W., Goussi, R., Koyro, H. W., Abdelly, C. & Manaa, A. Physiological and biochemical markers for screening salt tolerant quinoa genotypes at early seedling stage. *J. Plant Interact.* **15**, 27–38. <https://doi.org/10.1080/17429145.2020.1722266> (2020).
10. Manaa, A. et al. Salinity tolerance of quinoa (*Chenopodium quinoa* Willd.) as assessed by chloroplast ultrastructure and photosynthetic performance. *Environ. Exp. Bot.* **162**, 103–114. <https://doi.org/10.1016/j.envexpbot.2019.02.012> (2019).
11. Manaa, A. et al. Photosynthetic performance of quinoa (*Chenopodium quinoa* Willd.) after exposure to a gradual drought stress followed by a recovery period. *Biochim. et Biophys. Acta (BBA) – Bioenerg.* **1862**, 148383. <https://doi.org/10.1016/j.bbabi.2021.148383> (2021).
12. Jacobsen, S. E., Liu, F. & Jensen, C. R. Does root-sourced ABA play a role for regulation of stomata under drought in quinoa (*Chenopodium quinoa* Willd.). *Sci. Hortic.* **122**, 281–287. <https://doi.org/10.1016/j.scienta.2009.05.019> (2009).
13. Jacobsen, S. E. et al. Frost resistance mechanisms in quinoa (*Chenopodium quinoa* Willd.). *Eur. J. Agron.* **26**, 471–475. <https://doi.org/10.1016/j.eja.2007.01.006> (2007).
14. Zurita Silva, A. et al. Quinoa drought responses and adaptation. In *State of the Art Report on Quinoa Around the World in 2013* (eds Bazile, D. et al.) 157–171 (FAO/CIRAD, 2015).
15. Repo-Carrasco, R., Espinoza, C. & Jacobsen, S. E. Nutritional value and use of the Andean crops quinoa (*Chenopodium quinoa*) and Kañiwa (*Chenopodium pallidicaule*). *Food Rev. Int.* **19**, 179–189. <https://doi.org/10.1081/FRI-120018884> (2003).
16. Vega-Gálvez, A. et al. Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* Willd.), an ancient Andean grain: A review. *J. Sci. Food Agric.* **90**, 2541–2547. <https://doi.org/10.1002/jsfa.4158> (2010).
17. Nowak, V., Du, J. & Charrondière, U. R. Assessment of the nutritional composition of quinoa (*Chenopodium quinoa* Willd.). *Food Chem.* **193**, 47–54. <https://doi.org/10.1016/j.foodchem.2015.02.111> (2016).
18. Bazile, D., Jacobsen, S. E. & Verniau, A. The global expansion of quinoa: trends and limits. *Front. Plant Sci.* **7**, 622. <https://doi.org/10.3389/fpls.2016.00622> (2016).
19. Iqbal, H., Yaning, C., Waqas, M., Shareef, M. & Raza, S. T. Differential response of quinoa genotypes to drought and foliage-applied H₂O₂ in relation to oxidative damage, osmotic adjustment and antioxidant capacity. *Ecotoxicol. Environ. Saf.* **164**, 344–354. <https://doi.org/10.1016/j.ecoenv.2018.08.004> (2018).
20. Razzaghi, F., Jacobsen, S. E., Jensen, C. R. & Andersen, M. N. Ionic and photosynthetic homeostasis in quinoa challenged by salinity and drought: Mechanisms of tolerance. *Funct. Plant Biol.* **42**, 136–148. <https://doi.org/10.1071/FP14132> (2015).
21. Ricardo, A., Nguyen, A., Santiago, P., Richard, J. & Thierry, W. Rooting plasticity in wild and cultivated Andean *Chenopodium* species under soil water deficit. *Plant Soil* **425**, 479–492. <https://doi.org/10.1007/s11104-018-3588-7> (2018).
22. Jensen, C. R. et al. Leaf gas exchange and water relation characteristics of field quinoa (*Chenopodium quinoa* Willd.) during soil drying. *Eur. J. Agron.* **13**, 11–25. [https://doi.org/10.1016/S1161-0301\(00\)00055-1](https://doi.org/10.1016/S1161-0301(00)00055-1) (2000).
23. Goussi, R. et al. Comparative analysis of salt stress, duration and intensity, on the chloroplast ultrastructure and photosynthetic apparatus in *Thellungiella salsuginea*. *J. Photochem. Photobiol. B Biol.* **183**, 275–287. <https://doi.org/10.1016/j.jphotobiol.2018.04.047> (2018).
24. Huan, X. et al. Integrating transcriptomics and metabolomics to analyze quinoa (*Chenopodium quinoa* Willd.) responses to drought stress and rewatering. *Front. Plant Sci.* **13**, 988861. <https://doi.org/10.3389/fpls.2022.988861> (2022).
25. Zou, J. et al. Screening of drought resistance indices and evaluation of drought resistance in cotton (*Gossypium hirsutum* L.). *J. Integr. Agric.* **19**, 495–508. [https://doi.org/10.1016/S2095-3119\(19\)62696-1](https://doi.org/10.1016/S2095-3119(19)62696-1) (2020).
26. Miranda-Apodaca, J., Yoldi-Achalandabaso, A. & Aguirresarobe, A. Similarities and differences between the responses to osmotic and ionic stress in quinoa from a water use perspective. *Agric. Water Manag.* **203**, 344–352. <https://doi.org/10.1016/j.agwat.2018.03.026> (2018).
27. Volkov, V., Wang, B., Dominy, P. J., Fricke, W. & Amtmann, A. *Thellungiella halophila*, a salt-tolerant relative of *Arabidopsis thaliana*, possesses effective mechanisms to discriminate between potassium and sodium. *Plant Cell Environ.* **27**, 1–14. <https://doi.org/10.1046/j.0016-8025.2003.01116.x> (2004).
28. Nobel, P. S. *Physicochemical and Environmental Plant Physiology* (Academic Press, 1991).
29. Faurobert, M., Pelpoir, E. & Chaïb, J. Phenol Extraction of Proteins for Proteomic Studies of Recalcitrant Plant Tissues. In *Plant Proteomics Methods in Molecular Biology* (eds Thiellement, H. et al.) 9–14 (Humana Press, 2007).
30. Ramagli, L. S. & Rodriguez, L. V. Quantitation of microgram amounts of protein in two-dimensional polyacrylamide gel electrophoresis sample buffer. *Electrophoresis* **6**, 559–563. <https://doi.org/10.1002/elps.1150061109> (1985).
31. Cordara, A. et al. Response of the thylakoid proteome of *Synechocystis* sp. PCC 6803 to photoinhibitory intensities of orange-red light. *Plant Physiol. Biochem.* **132**, 524–534. <https://doi.org/10.1016/j.plaphy.2018.10.002> (2018).
32. Goussi, R. et al. Thylakoid proteome variation of *Eutrema salsugineum* in response to drought and salinity combined stress. *BBA Bioenerg.* **1862**, 148482. <https://doi.org/10.1016/j.bbabi.2021.148482> (2021).
33. Guo, X. & Kristal, B. S. The use of underloaded C18 solid-phase extraction plates increases reproducibility of analysis of tryptic peptides from unfractionated human plasma. *Anal. Biochem.* **426**, 86–90. <https://doi.org/10.1016/j.ab.2012.04.003> (2012).
34. Helmann, U., Wernstedt, C., Gonez, J. & Heldin, C. Improvement of an “In-gel” digestion procedure for the micropreparation of internal protein fragments for amino acid sequencing. *Anal. Biochem.* **224**, 451–455. <https://doi.org/10.1006/abio.1995.1070> (1995).
35. Albanese, P. et al. Thylakoid proteome modulation in pea plants grown at different irradiances: Quantitative proteomic profiling in a non-model organism aided by transcriptomic data integration. *Plant J.* **96**, 786–800. <https://doi.org/10.1111/tpl.14068> (2018).
36. Gillet, L. et al. Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: A new concept for consistent and accurate proteome analysis. *Mol. Cell. Proteomics* **11**, O111.016717. <https://doi.org/10.1074/mcp.O111.016717> (2012).
37. Venable, J. D., Dong, M. Q., Wohlschlegel, J., Dillin, A. & Yates, J. R. Automated approach for quantitative analysis of complex peptide mixtures from tandem mass spectra. *Nat. Methods* **1**, 39–45. <https://doi.org/10.1038/nmeth705> (2004).
38. Begna, T. Impact of drought stress on crop production and its management options. *Int. J. Res. Agron.* **8**, 1–13. <https://doi.org/10.12043/2454-6224.0812001> (2022).
39. Ahmad, H. M., Wang, X., Fiaz, S., Azeem, F. & Shaheen, T. Morphological and physiological response of *Helianthus annuus* L. to drought stress and correlation of wax contents for drought tolerance traits. *Arab. J. Sci. Eng.* **47**, 6747–6761. <https://doi.org/10.1007/s13369-021-06098-1> (2022).
40. Babar, M. et al. A comprehensive review on drought stress response in cotton at physiological, biochemical and molecular level. *Pure Appl. Biol.* **12**, 610–622. <https://doi.org/10.19045/bspab.2023.120063> (2023).
41. Shiade, S. R. G., Fathi, A., Taghavi Ghasemkheili, F., Amir, E. & Pessarakli, M. Plants’ responses under drought stress conditions: Effects of strategic management approaches—A review. *J. Plant Nutr.* **46**, 2198–2230. <https://doi.org/10.1080/01904167.2022.2105720> (2022).
42. Berauer, B. J., Steppuhn, A. & Schweiger, A. H. The multidimensionality of plant drought stress: The relative importance of edaphic and atmospheric drought. *Plant Cell Environ.* **47**, 3528–3540. <https://doi.org/10.1111/pce.15012> (2024).

43. Mirsafi, S. M., Sepaskhah, A. R. & Ahmadi, S. H. Physiological traits, crop growth, and grain quality of quinoa in response to deficit irrigation and planting methods. *BMC Plant Biol.* **24**, 809. <https://doi.org/10.1186/s12870-024-05523-5> (2024).
44. Zahedi, S. M. et al. Plant adaptation to drought stress: The role of anatomical and morphological characteristics in maintaining the water status. *J. Soil Sci. Plant Nutr.* **25**, 409–427. <https://doi.org/10.1007/s42729-024-02141-w> (2025).
45. An, Y., Zhang, M., Liu, G., Han, R. & Liang, Z. Proline accumulation in leaves of *Periploca sepium* via both biosynthesis up-regulation and transport during recovery from severe drought. *PLoS ONE* **8**, e69942. <https://doi.org/10.1371/journal.pone.0069942> (2013).
46. Wang, Y. et al. Proteomic analysis of *Camellia sinensis* (L.) reveals a synergistic network in the response to drought stress and recovery. *J. Plant Physiol.* **219**, 91–99. <https://doi.org/10.1016/j.jplph.2017.10.001> (2017).
47. Bascuñán-Godoy, L., Reguera, M., Abdel-Tawab, Y. M. & Blumwald, E. Water deficit stress-induced changes in carbon and nitrogen partitioning in *Chenopodium quinoa* Willd. *Planta* **243**, 591–603. <https://doi.org/10.1007/s00425-015-2424-z> (2016).
48. Jongdee, B., Fukai, S. & Cooper, M. Leaf water potential and osmotic adjustment as physiological traits to improve drought tolerance in rice. *Field Crops Res.* **76**, 153–163. [https://doi.org/10.1016/S0378-4290\(02\)00036-9](https://doi.org/10.1016/S0378-4290(02)00036-9) (2002).
49. Akram, M. Z., Libutti, A. & Rivelli, A. R. Evaluation of vegetative development of quinoa under water stress by applying different organic amendments. *Agronomy* **13**, 1412. <https://doi.org/10.3390/agronomy13051412> (2023).
50. Chen, M. X., Zhang, Y., Fernie, A. R., Liu, Y. G. & Zhu, F. Y. SWATH-MS-based proteomics: Strategies and applications in plants. *Trends Biotechnol.* **39**, 433–437. <https://doi.org/10.1016/j.tibtech.2020.09.002> (2021).
51. Graça, I. et al. Comparative proteomic analysis of nodulated and non-nodulated *Casuarina glauca* Sieb. ex Spreng. grown under salinity conditions using sequential window acquisition of all theoretical mass spectra (SWATH-MS). *Int. J. Mol. Sci.* **21**, 78. <https://doi.org/10.3390/ijms21010078> (2020).
52. Cheng, X. et al. Protein expression changes in response to potassium deficiency stress of industrial hemp plants using SWATH technology. *Ind. Crops Prod.* **174**, 114190 (2021).
53. Ganie, S. A. & Ahammed, G. J. Dynamics of cell wall structure and related genomic resources for drought tolerance in rice. *Plant Cell Rep.* **40**, 437–459. <https://doi.org/10.1007/s00299-020-02649-2> (2021).
54. Chaves, M. M., Maroco, J. P. & Pereira, J. S. Understanding plant responses to drought—from genes to the whole plant. *Funct. Plant Biol.* **30**, 239–264. <https://doi.org/10.1071/FP02076> (2003).
55. Wasteneys, G. O. & Galway, M. E. Remodeling the cytoskeleton for growth and form: An overview with some new views. *Annu. Rev. Plant Biol.* **54**, 691–722. <https://doi.org/10.1146/annurev.arplant.54.031902.134818> (2003).
56. Han, X. et al. Lignin biosynthesis and accumulation in response to abiotic stresses in woody plants. *For Res. (Fayettev)* **2**, 9. <https://doi.org/10.48130/FR-2022-0009> (2022).
57. Wang, X., Cai, X., Xu, C., Wang, Q. & Dai, S. Drought-responsive mechanisms in plant leaves revealed by proteomics. *Int. J. Mol. Sci.* **17**, 1706. <https://doi.org/10.3390/ijms17101706> (2016).
58. Zhao, D. et al. A *Paeonia ostii* caffeoyl-CoA O-methyltransferase confers drought stress tolerance by promoting lignin synthesis and ROS scavenging. *Plant Sci.* **303**, 110765. <https://doi.org/10.1016/j.plantsci.2020.110765> (2021).
59. Li, C., Qi, Y., Zhao, C., Wang, X. & Zhang, Q. Transcriptome profiling of the salt stress response in the leaves and roots of halophytic *Eutrema salsugineum*. *Front. Genet.* **12**, 770742. <https://doi.org/10.3389/fgene.2021.770742> (2021).
60. Huang, S. et al. MsCCoAOMT3 confers drought tolerance by mediating lignin content and ROS scavenging. *Plant Mol. Biol.* **116**, 8. <https://doi.org/10.1007/s11103-025-01674-7> (2026).
61. Sniegowska-Swierk, K., Dubas, E. & Rapacz, M. Drought-induced changes in the actin cytoskeleton of barley (*Hordeum vulgare* L.) leaves. *Acta Physiol. Plant.* **37**, 73. <https://doi.org/10.1007/s11738-015-1820-0> (2015).
62. Wu, Y., Mirzaei, M., Pascovici, D., Haynes, P. A. & Atwell, B. J. Proteomes of leaf-growing zones in rice genotypes with contrasting drought tolerance. *Proteomics* **19**, 1800310. <https://doi.org/10.1002/pmic.201800310> (2019).
63. Yin, H. et al. Advances in the functional study of glutamine synthetase in plant abiotic stress tolerance response. *Crop J.* **10**, 917–923. <https://doi.org/10.1016/j.cj.2022.01.003> (2022).
64. Žárský, V. Exocyst functions in plants: Secretion and autophagy. *FEBS Lett.* **596**, 2324–2334. <https://doi.org/10.1002/1873-3468.14430> (2022).
65. Boo, Y. C. & Jung, J. Water deficit—induced oxidative stress and antioxidative defenses in rice plants. *J. Plant Physiol.* **155**, 255–261. [https://doi.org/10.1016/S0176-1617\(99\)80016-9](https://doi.org/10.1016/S0176-1617(99)80016-9) (1999).
66. Singh, A. & Satheeshkumar, P. K. Reactive oxygen species (ROS) and ROS scavengers in plant abiotic stress response. In *Stress Biology in Photosynthetic Organisms* (ed. Mishra, A. K.) 41–63 (Springer, 2024). https://doi.org/10.1007/978-981-97-1883-2_3.
67. Chinmayi, S., Yadav, M., Nandhan, N. S., Barot, M., Saikrishna, Enzymatic and nonenzymatic antioxidant defense in plants under drought stress. In Husen A (ed) *Plant biology, sustainability and climate change: role of antioxidants in mitigating plant stress*. Academic Press, pp 75–85. (2025) <https://doi.org/10.1016/B978-0-443-26799-4.00016-5>
68. Hazra, S. et al. Nucleoredoxin vis-à-vis a novel thioredoxin in regulating oxidative stress in plants: A review. *Agric. Res.* **13**, 400–418. <https://doi.org/10.1007/s40003-024-00737-3> (2024).
69. Lau, S. E. et al. Combined proteomics and physiological analyses reveal drought and recovery response mechanisms in banana leaves. *J. Plant Growth Regul.* **42**, 7624–7648. <https://doi.org/10.1007/s00344-023-11039-3> (2023).
70. Sofo, A., Tuzio, A. C., Dichio, B. & Xiloyannis, C. Influence of water deficit and rewetting on the components of the ascorbate–glutathione cycle in four interspecific *Prunus* hybrids. *Plant Sci.* **169**, 403–412. <https://doi.org/10.1016/j.plantsci.2005.04.004> (2005).
71. Furlan, A., Bianucci, E., Tordable, M., Castro, S. & Dietz, K. J. Antioxidant enzyme activities and gene expression patterns in peanut nodules during a drought and rehydration cycle. *Funct. Plant Biol.* **41**, 704–713. <https://doi.org/10.1071/FP13311> (2014).
72. Singh, S. K. et al. Molecular chaperones: a key player for combating the effect of abiotic stresses. In *Stress tolerance in horticultural crops* (eds Chandra Rai, A. et al.) 155–168 (Woodhead Publishing, 2021).
73. Kumar, P. et al. Understanding heat-shock proteins' abundance and pivotal function under multiple abiotic stresses. *J. Plant Biochem. Biotechnol.* **33**, 492–513. <https://doi.org/10.1007/s13562-024-00932-x> (2024).
74. Demirevska, K. et al. Drought-induced leaf protein alterations in sensitive and tolerant wheat varieties. *Gen. Appl. Plant Physiol.* **34**, 79–102 (2008).
75. Timperio, A. M., Egidi, M. G. & Zolla, L. Proteomics applied on plant abiotic stresses: Role of heat shock proteins (HSP). *J. Proteom.* **71**, 391–411. <https://doi.org/10.1016/j.jprot.2008.07.005> (2008).
76. Çulha Erdal, Ş., Eyidoğan, F. & Ekmekçi, Y. Comparative physiological and proteomic analysis of cultivated and wild safflower response to drought stress and re-watering. *Physiol. Mol. Biol. Plants.* **27**, 281–295. <https://doi.org/10.1007/s12298-021-00934-2> (2021).
77. Parry, M. A. J. et al. Rubisco activity: Effects of drought stress. *Ann. Bot.* **89**, 833–839. <https://doi.org/10.1093/aob/mcf103> (2002).
78. Demirevska, K. et al. Drought stress effects on Rubisco in wheat: Changes in the Rubisco large subunit. *Acta Physiol. Plant.* **31**, 1129–1138. <https://doi.org/10.1007/s11738-009-0331-2> (2009).
79. Brossa, R. et al. Redox proteomics and physiological responses in *Cistus albidus* shrubs subjected to long-term summer drought followed by recovery. *Planta* **241**, 803–822. <https://doi.org/10.1007/s00425-014-2221-0> (2015).
80. Rolly, N. K. & Yun, B. W. Regulation of nitrate (NO₃) transporters and glutamate synthase-encoding genes under drought stress in *Arabidopsis*: The regulatory role of AtbZIP62 transcription factor. *Plants* **10**, 2149. <https://doi.org/10.3390/plants10102149> (2021).

81. Takabayashi, A., Niwata, A. & Tanaka, A. Direct interaction with ACR11 is necessary for post-transcriptional control of GLU1-encoded ferredoxin-dependent glutamate synthase in leaves. *Sci. Rep.* **6**, 29668. <https://doi.org/10.1038/srep29668> (2016).
82. Budak, H., Akpinar, B. A., Unver, T. & Turktas, M. Proteome changes in wild and modern wheat leaves upon drought stress by two-dimensional electrophoresis and nanoLC-ESI-MS/MS. *Plant Mol. Biol.* **83**, 89–103. <https://doi.org/10.1007/s11103-013-0024-5> (2013).
83. Vannini, C. et al. Proteomic analysis reveals how pairing of a mycorrhizal fungus with plant growth-promoting bacteria modulates growth and defense in wheat. *Plant Cell Environ.* **44**, 1946–1960. <https://doi.org/10.1111/pce.14039> (2021).
84. Shu, L. et al. Genetic, proteomic and metabolic analysis of the regulation of energy storage in rice seedlings in response to drought. *Proteomics* **11**, 4122–4138. <https://doi.org/10.1002/pmic.201000485> (2011).
85. Kumari, A., Das, P., Parida, A. K. & Agarwal, P. K. Proteomics, metabolomics, and ionomics perspectives of salinity tolerance in halophytes. *Front. Plant Sci.* **6**, 537. <https://doi.org/10.3389/fpls.2015.00537> (2015).
86. Azri, W. et al. Physiological and proteomic analyses of Tunisian local grapevine (*Vitis vinifera*) cultivar Razegui in response to drought stress. *Funct. Plant Biol.* **49**, 25–39. <https://doi.org/10.1071/FP21026> (2021).
87. Martins, R. D. S. et al. Proteomic analyses unraveling water stress response in two *Eucalyptus* species originating from contrasting environments for aridity. *Mol. Biol. Rep.* **47**, 5191–5205. <https://doi.org/10.1007/s11033-020-05594-1> (2020).
88. Chintakovid, N. et al. Proteomic analysis of drought-responsive proteins in rice reveals photosynthesis-related adaptations to drought stress. *Acta Physiol. Plant.* **39**, 240. <https://doi.org/10.1007/s11738-017-2532-4> (2017).
89. Derbali, W. et al. Comparative proteomic approach to study the salinity effect on the growth of two contrasting quinoa genotypes. *Plant Physiol. Biochem.* **163**, 215–229. <https://doi.org/10.1016/j.plaphy.2021.03.055> (2021).
90. Ashoub, A., Beckhaus, T., Berberich, T., Karas, M. & Brüggemann, W. Comparative analysis of barley leaf proteome as affected by drought stress. *Planta* **237**, 771–781. <https://doi.org/10.1007/s00425-012-1798-4> (2013).
91. Batista-Silva, W. et al. The role of amino acid metabolism during abiotic stress release. *Plant Cell Environ.* **42**(5), 1630–1644. <https://doi.org/10.1111/pce.13518> (2019).
92. Osanai, T., Kuwahara, A., Otsuki, H., Saito, K. & Hirai, M. Y. ACR11 is an activator of plastid-type glutamine synthetase GS2 in *Arabidopsis thaliana*. *Plant Cell Physiol.* **58**, 650–657. <https://doi.org/10.1093/pcp/pcx033> (2017).
93. Singh, S. K. et al. ACR11 modulates levels of reactive oxygen species and salicylic acid-associated defense response in *Arabidopsis*. *Sci. Rep.* **8**, 11851. <https://doi.org/10.1038/s41598-018-30304-0> (2018).
94. Azri, W. et al. Physiological and proteomic responses to drought stress in leaves of two wild grapevines (*Vitis sylvestris*): A comparative study. *Plant Growth Regul.* **91**, 37–52. <https://doi.org/10.1007/s10725-020-00586-4> (2020).
95. Yang, C. et al. SpBADH of the halophyte *Sesuvium portulacastrum* strongly confers drought tolerance through ROS scavenging in transgenic *Arabidopsis*. *Plant Physiol. Biochem.* **96**, 377–387. <https://doi.org/10.1016/j.plaphy.2015.08.010> (2015).
96. Ashraf, M. & Foolad, M. R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* **59**, 206–216. <https://doi.org/10.1016/j.envexpbot.2005.12.006> (2007).
97. Lin, C. C. & Kao, C. H. Regulation of ammonium-induced proline accumulation in detached rice leaves. *Plant Growth Regul.* **35**, 69–74. <https://doi.org/10.1023/A:1013832630830> (2001).
98. Damaris, R. N. et al. A proteomic analysis of salt stress response in seedlings of two African rice cultivars. *BBA Proteins Proteomics* **1864**, 1570–1578. <https://doi.org/10.1016/j.bbapap.2016.08.011> (2016).
99. Kim, K. Y. et al. Molecular cloning of low-temperature-inducible ribosomal proteins from soybean. *J. Exp. Bot.* **55**, 1153–1155. <https://doi.org/10.1093/jxb/erh125> (2004).
100. Shan, Z. et al. Physiological and proteomic analysis on long-term drought resistance of cassava (*Manihot esculenta* Crantz). *Sci. Rep.* **8**, 17982. <https://doi.org/10.1038/s41598-018-35711-x> (2018).
101. Mohammadi, P. P., Moieni, A. & Komatsu, S. Comparative proteome analysis of drought-sensitive and drought-tolerant rapeseed roots and their hybrid F1 line under drought stress. *Amino Acids* **43**, 2137–2152. <https://doi.org/10.1007/s00726-012-1299-6> (2012).

Acknowledgements

Authors acknowledge the United States Department of Agriculture (USDA) for providing quinoa seeds. The authors acknowledge Mohamed Ben Salem, for taking care of the plants.

Author contributions

All authors cited in the manuscript have made a significant contribution to the paper: Rahma Goussi: Plant culture, statistical analysis and manuscript preparation; Simone Cantamessa: Plant growth determination, Protein extraction and immunoblotting; Marcello Manfredi and Emilio Marengo: Mass spectrometric analysis; Arafet Manaa and Roberto Barbato: Experimentation plan and supervision of work. All authors read and approved the final versions of the manuscript.

Funding

The authors declare the following financial interests: This research was supported by the projects “Quinoa4Med” and “Purecircles” which are part of the Partnership on Research and Innovation in the Mediterranean Area (PRIMA, Grant Agreement no. 101102316) Program supported by the European Union and funded by the national funding bodies of Participating States (MHESR in Tunisia).

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-026-51620-w>.

Correspondence and requests for materials should be addressed to A.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2026