RESEARCH ARTICLE

Comparative physiological and proteomic analysis of cultivated and wild safflower response to drought stress and re-watering

Şeküre Çulha Erdal[1] · Füsun Eyidoğan[2] · Yasemin Ekmekçi[1]

Abstract Drought is one of the major environmental stress that adversely affect the growth and development of oil seed plant, safflower. There is a limited knowledge on proteomic responses to support physiological, biochemical changes in how safflowers can regulate growth and metabolism under drought conditions and followed by re-watering. The changes in morphological, physiological, biochemical and proteomics of safflower genotypes (*Carthamus tinctorius* L.; Remzibey-05 and Linas, tolerant and sensitive cultivars, respectively, and *C. oxyacantha* M. Bieb., wild type) after exposure to drought and followed by re-watering have been examined. Drought negatively affected the shoot weight, water content, chlorophyll fluorescence, and biochemical parameters, including photosynthetic pigment, proline, MDA, and H_2O_2 contents and antioxidant enzyme activities in all genotypes, while the re-watering period allowed Remzibey-05 to recover, and it even provided the wild type completely recovered (approximately 100%). A total of 72 protein spots were observed as differently accumulated under treatments. The identified proteins were mainly involved in photosynthesis and carbohydrate, protein, defense, and energy metabolisms. Protein accumulation related to these metabolisms in Remzibey-05 were decreased under drought, while increased following re-

watering. However, sensitive cultivar, Linas, could not exhibit an effective performance under drought and recovery when compared with other safflower genotypes.

Keywords *Carthamus oxyacantha* M. Bieb · Drought · Morpho-physiological and biochemical traits · Proteomics · RT-qPCR · Safflower

✉ Yasemin Ekmekçi
yase@hacettepe.edu.tr; ekmekciy@gmail.com

[1] Faculty of Science, Department of Biology, Hacettepe University, 06800 Ankara, Turkey

[2] Faculty of Education, Department of Elementary Education, Başkent University, 06810 Ankara, Turkey

Introduction

Drought is one of the most important factors induced by global climate change which reduces the amount of usable water, affects especially agricultural areas and causes significant yield losses in agricultural products. Therefore, the importance of irrigation increases every day; however, the resources used for agricultural irrigation are gradually decreasing in many regions of the world. Intergovernmental Panel on Climate Change (IPCC) reported that the water level in dry subtropic regions will be reduced further and the existing water cannot be used due to contamination in the twenty-first century (IPCC 2019).

Drought is a multidimensional stress factor and affects the plant at the cell, organ and organism levels. Under water-deficient conditions, the plant-water relationship is disrupted and eventually, the cell water potential and turgor are reduced (Taiz et al. 2015). The first response to the reduction of water in the plant is a reduction in photosynthesis which involves the closure of stomata and decrease in the activity of photosynthetic enzymes and ATP synthesis (Farooq et al. 2009). Another drought-induced effect exacerbates the synthesis of reactive oxygen species (ROS) to levels that are beyond the plant's detoxification capacity, and thus trigger oxidative stress. These stress-induced changes may also interfere with plant

growth and development by affecting other metabolic activities such as respiration, transport and carbohydrate metabolism. Plants regulate their adaptation strategies against drought by adjusting morphology, physiology and biochemical activities including control of water content, developing deep root systems, and accumulation of osmolytes and activating enzyme and non-enzyme systems to cope with oxidative stresses (Xin et al. 2018).

It has been reported that knowledge about molecular mechanisms generated to understand drought responses shows all stages from starting signal detection to the end product of post-translational protein modifications (PTMs) cannot be revealed by genomic or transcriptomic analysis (Nemati et al. 2019). The use of related technologies for the identification of proteins and PTMs and the detection of expression profile and protein–protein interactions facilitate research on the effects of plant development. Proteomics studies provide information about the actual fluctuations of the protein levels associated with physiological and stress responses (Budak et al. 2015; Urban et al. 2017; Nemati et al. 2019; Azra et al. 2020).

Safflower (Carthamus tinctorius L.) is one of the oldest oilseed plants in the world that has high omega-9 (16–20% oleic acid) and omega-6 (71–75% linoleic acid) content. Safflower is used mainly for the production of vegetable oil, and also used in medical, cosmetic and paint industries (Gecgel et al. 2007). Besides, safflower oil has been reported to be suitable for biodiesel production (Mihaela et al. 2013). Although, safflower has a narrow cultivation area in the world, it could be one of the important alternative oil plants especially in arid and semi-arid regions if its tolerance to stress factors such as drought can be proved (Majidi et al. 2011). The vegetative stage is reported as the most critical stage (especially before flowering) for safflower (Istanbulluoglu et al. 2009). Therefore, research on the effect of drought on safflower at this critical stage which reflects yield and production is very important. The current study aimed to determine the drought tolerance capacity of safflower cultivars and to compare the responses of these cultivars with the wild safflower Carthamus oxyacantha. To achieve this, safflower genotypes at the vegetative stage were characterized by morphological, physiological and biochemical changes, and differentially expressed protein (DEP) profiles were identified using proteomics analysis.

Materials and methods

Plant growth and treatments

Safflower (Carthamus tinctorius L.) cultivars Remzibey-05 and Linas were selected for this study according to the previous results (Çulha-Erdal et al. 2016), where Remzibey-05 and Linas were identified as tolerant and sensitive, respectively. Besides, wild type safflower genotype (Carthamus oxyacantha M.Bieb.) was used in the study. Seeds, obtained from Trakya Agricultural Research Institute, Turkey (TARI), were surface-sterilized (5% NaClO for 3 min) and imbibed in distilled water for 2 h. After incubation, seeds were sown in PVC pots holding 800 g airdried soil with 19.5% water holding capacity. Some characteristics of soil were clay loam texture, pH 7.52 and electrical conductivity 264 μS cm^{-1}. Each pot contained four plants. Plants were grown in a controlled growth chamber with a temperature of 25 ± 1 °C, 16 h photoperiod, $40 \pm 5\%$ air humidity and 250 μmol m^{-2} s^{-1} light intensity. On the 40th day after sowing, pots of each genotype were randomly divided into two groups, one of which served as the control which was irrigated regularly, while the other was subjected to drought without irrigation for 7 days followed by 5 days of re-watering. Plants were harvested at the beginning of drought (C-40th day) and, at the end of drought (S-47th day) and after re-watering (R – 52nd day) periods to provide suitable analysis.

Morphological, physiological and biochemical analysis

Three plants representing each treatment were harvested to determine fresh weights. Subsequently, dry weights of plants were measured by drying the fresh plants at 80 °C for 48 h. The water status of the leaves was evaluated by calculating relative water content (RWC) (Farrant 2000).

Following at least 30 min dark adaptation, the minimum (Fo) and maximum (F_M) chlorophyll a fluorescence (ChlF) were measured. The maximal quantum efficiency of PSII of dark-adapted plants (F_V/F_M) was calculated using (F_M-F_O)/F_M. The efficiency of the water-splitting complex on the donor side of PSII (F_V/F_O) was also calculated to assess PSII activity. Light induced changes in ChlF, following actinic illumination (300 μmol m^{-2} s^{-1}) were recorded prior to the measurement of minimum (F_O') and maximum (F_M') fluorescence in light-adapted state. The quantum efficiency of PSII open centers in the light-adapted state, referred to as ΦPSII, (F_M'- F_S/F_M') was determined from F'_M and Fs (steady-state fluorescence in the light-saturated state) values. The quantum efficiency of excitation energy trapping of PSII (F_V'/F_M') was calculated according to Genty et al. (1989). Later, actinic light was closed and F_O' was determined by illuminating the leaves with far-red light (7 μmol m^{-2} s^{-1}). Electron transport rate (ETR) was also calculated by the following formula: (F_M'-F_S)/(F_M-) × PAR × 0.84 × 0.5 (Genty et al. 1989). Non-photochemical quenching NPQ = F_M-F_M $'/F_M'$ were also calculated according to Bilger and Björkman (1990). The

fraction of open PSII reaction centers as $q_L = (F_M'-F_S)/(F_M'-F_O') \times (F_O'-F_S)$ were calculated according to Kramer et al. (2004).

For each treatment, photosynthetic pigment [chlorophyll (a + b) and carotenoids (x + c)] contents were determined from leaf disks (R = 0.6 cm) and calculated as mg ml^{-1} cm^{-2} according to Lichtenthaler (1987). The anthocyanin content (mg ml^{-1} g FW^{-1}) was measured as described by Mancinelli et al. (1975).

The free proline content (µmol g FW^{-1}) was determined using the method of Bates et al. (1973) and a proline standard curve was used to determine the content of free proline. The levels of malondialdehyde (MDA) and hydrogen peroxide (H$_2$O$_2$) content were measured according to the method of Esterbauer and Cheeseman (1990). MDA content (nmol g FW^{-1}) was calculated using the extinction coefficient 155 mM^{-1} cm^{-1}. H$_2$O$_2$ content (µmol g FW^{-1}) was calculated according to the standard curve.

Fresh leaf samples (0.5 g) were grounded with liquid nitrogen and soluble proteins were extracted by the related homogenizing buffer. In the leaf extracts, the protein concentrations were determined according to Bradford (1976). Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined according to Beyer and Fridovich (1987). One unit of SOD is defined as the amount of enzyme which causes 50% decrease of the SOD-inhibited NBT reduction. Ascorbate peroxidase (APX; EC 1.11.1.11) activity was assayed according to the method of Wang et al. (1991) and the enzyme activity was calculated using $\varepsilon = 2.8$ mM cm^{-1} at 290 nm. Glutathione reductase (GR; EC 1.6.4.2) activity was determined according to Rao et al. (1995). The enzyme activity was calculated after subtracting the non-enzymic initial oxidation rate using the extinction coefficient of NADPH ($\varepsilon = 6.2$ mM cm^{-1}) at 340 nm. Guaiacol peroxidase (POD; EC1.11.1.7) activity was based on the determination of guaiacol oxidation ($\varepsilon = 26.6$ mM cm^{-1}) at 470 nm by H$_2$O$_2$ (Bergmeyer 1974). Catalase (CAT; EC 1.11.1.6) activity was assayed according to the method of Change and Maehly (1955) and the enzyme activity was calculated from the initial rate of decomposition of H$_2$O$_2$ using $\varepsilon = 40$ mM cm^{-1} at 240 nm. Aldose reductase (ALR; EC 1.1.1.21) activities were determined as described by Mundree et al. (2000). The enzyme activity was calculated from the initial rate of the reaction after subtracting the non-enzyme oxidation rate using the ε of NADPH ($\varepsilon = 6.2$ mM cm^{-1}) at 340 nm.

Proteomic analysis

Proteins were extracted using a phenol extraction method (Hurkman and Tanaka 1986) with modifications (Ahsan et al. 2008). Leaf tissues (2 g) were ground into a fine powder with liquid nitrogen and homogenized in 8 ml pre-cooled Mg/NP-40 extraction buffer (Kim et al. 2001). The homogenates were centrifuged at 3500 g for 15 min, and fractionated with an equal volume of Tris–HCl saturated phenol (pH 8.0). After that, the top phenol phase was collected, and proteins were precipitated overnight by adding four volumes of cold 0.1 M ammonium acetate in methanol at -20 °C. The precipitated proteins were collected by centrifugation at 10,800 g for 10 min and washed with precipitation solution three times and ice-cold acetone twice. The protein pellet was air-dried and dissolved in a rehydration buffer. Protein concentration was determined using the Ramagli and Rodriguez assay (1985).

The protein extracts were subjected to two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). 500 µg protein samples which were diluted with a 300 µl of rehydration buffer were loaded onto IPG (Immobilized pH gradient) strips (17 cm with 5–8 linear pH gradient; Bio-Rad, USA) and were passively rehydrated for 16 h. Isoelectric focusing (IEF) was carried out using PROTEAN®i12^{TM} IEF System (Bio-Rad, USA) at 20 °C with the following settings: 250 V for 30 min, 10,000 V for 2 h and finally 10,000 V per hour until reaching 70,000 V. After IEF, the strips were first incubated in equilibration buffer I (6 M urea, 2% SDS, 1,5 M Tris–HCl pH 8.8, 20% glycerol and 2% DTT) for 15 min and then in equilibration buffer II (6 M urea, 2% SDS, 1,5 M Tris–HCl pH 8.8, 20% glycerol and 2.5% iodoacetamide) in the following 15 min. The second dimension SDS–PAGE was performed on running gels (PROTEAN II XL Cell, Bio-Rad; 12.5% polyacrylamide) according to Laemmli (1970). The protein spots on gels were visualized by using Oriole^{TM} Fluorescent Gel Stain (Bio-Rad, USA). To assure reproducibility, the gels were run in 2-D PAGE three times in each experiment. Then, three of these gels obtained from each treatment were used for MALDI-TOF/TOF–MS analysis.

The stained gels were displayed using VERSADoc 4000 MP (BioRad, USA) system and images of the gels suitable for analysis were obtained using the Quantity One program (BioRad, USA). Analysis of protein spots and comparison of the gels was performed with PDQuest Advanced program (version 8.0.1, BioRad, USA). The statistical calculation of the difference in spots was conducted by Student's t-test ($P < 0.05$) and, changes more than two times were cut by using an automated spot cutting tool, ExQuest spot cutter (BioRad, USA).

Tryptic-digested peptides were extracted using an in-gel tryptic digestion kit (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer's protocol. Zip-Tip (Millipore, USA) cleaning was performed for each digested sample before deposition onto a MALDI plate. Protein identification was performed by using AB SCIEX MALDI-TOF/TOF 5800 system (Applied Biosystems, Framingham,

69MA, USA). Mass spectra (m/z 800–3000) were acquired in positive ion reflector mode. The 10 most intense ions were selected for subsequent MS/MS sequencing analysis in 1 kV mode. MS/MS spectra of proteins were submitted to database searching using the online MASCOT program (Matrix Science, London, UK) and GPS Explorer (Applied Biosystem).

To better understand interactions of identified proteins, the homolog of all identified proteins in *Arabidopsis thaliana* was determined using UniProt database, and STRING 11.0 database (http://string-db.org) was used to construct a network for protein–protein interactions (PPI). Besides, Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways have been used to schematize the drought-affected metabolic pathways.

Real-time PCR (RT-PCR) analysis

Total RNA was extracted from 0.3 g leaf samples according to Chomczynski and Sacchi (1987). RNA was purified using DNase I, RNase-free Set (Thermo Scientific, CA, USA) and Quantitation of RNA was obtained with a BioSpec-nano UV–VIS spectrophotometer (Shimadzu). The first strand cDNA was synthesized using Scientific RevertAid First-Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's instructions. Primers were designed using NCBI, Primer3 and IDT Oligo Analyzer programs (Supplementary Table S1). Expressions of genes were monitored by the real-time PCR cycler Rotor-Gene Q (Qiagen, Hilden, Germany) using Luminaris Color HiGreen qPCR Master Mix (Thermo Scientific). Elongation factor-1α (EF-1α) was used as internal control and data were normalized using the control samples.

Statistical analysis

Experiments were performed in a completely randomized design by three replicates (four plants in each replicate/pot). Differences between cultivars and treatments were analyzed by ANOVA and according to the least significant difference (LSD) test at the 5% level using the SPSS 20.0 software (IBM SPSS Statistics). qRT-PCR data were analyzed by student t-test ($p \leq 0.05$).

Results and Discussion

When exposed to environmental stress such as drought, plants develop complex mechanisms and re-program their metabolism and growth as induced by morphological, physiological, biochemical, and molecular changes (Claeys and Inzé 2013). In the present study, drought and re-watering periods caused significant changes in plant

morphology, physiology and biochemistry (Figs. 1, 2, 3, 4). Besides, our proteomic analysis showed that the water-stress-responsive proteins involved in photosynthesis and carbohydrate metabolism consist of the highest percentage of differentially expressed protein (DEPs) (Supplementary Table S2).

Characterization of drought tolerance by morphological, physiological and biochemical traits

In this study, the fresh weight of shoot decreased under drought stress and increased following re-watering in all genotypes (Fig. 1a). This reduction under drought might be associated with the suppression of cell expansion and growth due to a decrease in turgor pressure (Robert et al.
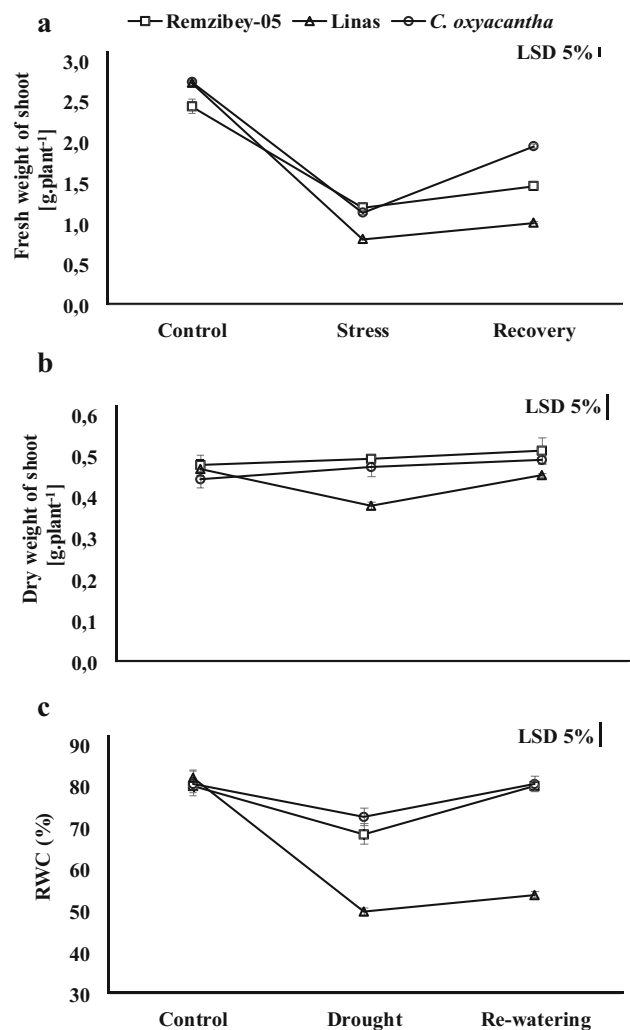

Fig. 1 Effect of drought and re-watering periods on fresh (**a**) and dry (**b**) weight of a plant and RWC = relative water content (**c**) in the leaves of safflower genotypes. The values are presented as the mean ± standard error
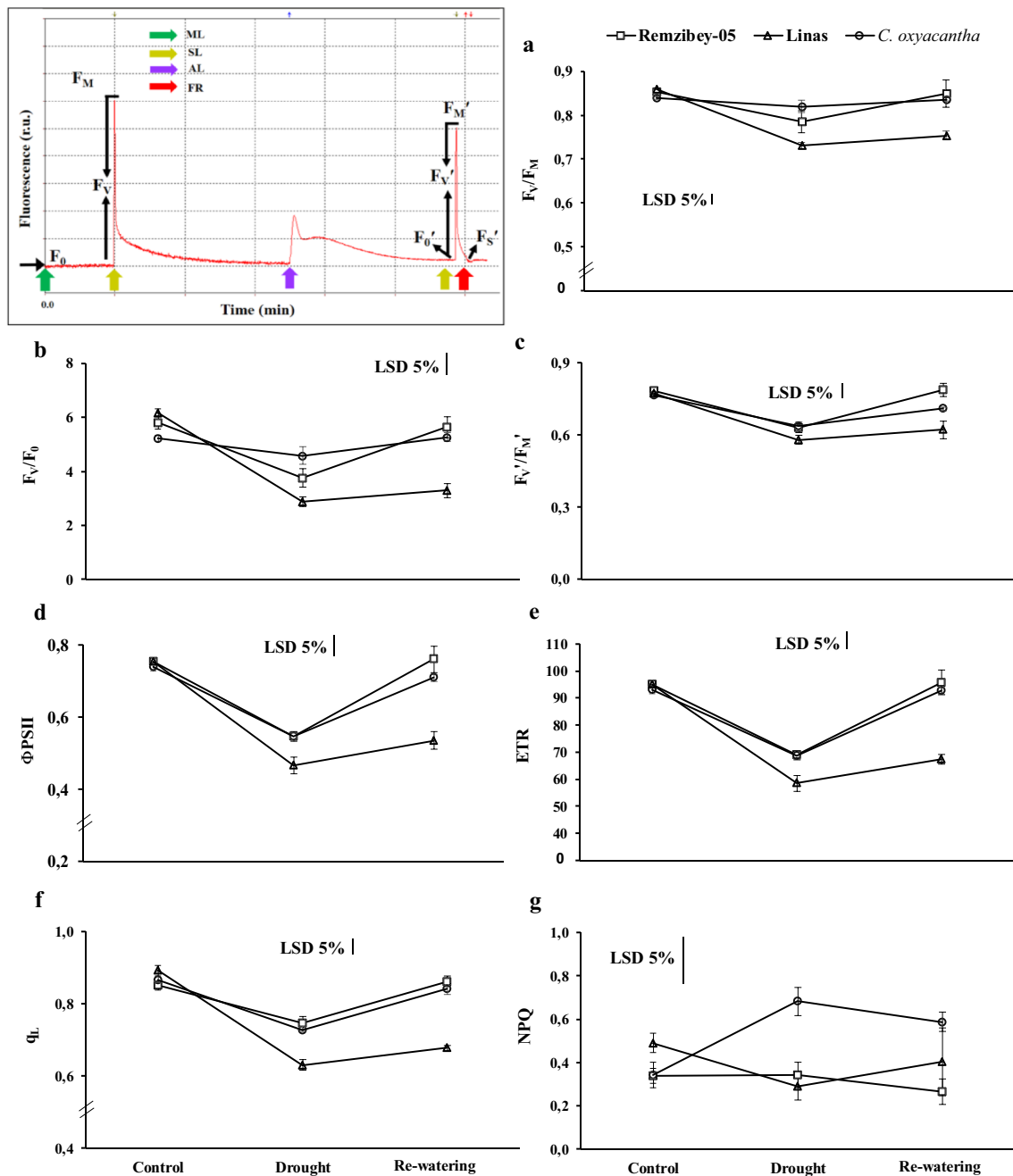
Fig. 2 Chlorophyll fluorescence responses of safflower leaves exposed to drought and re-watering periods: F_V/F_M = The maximal quantum efficiency of PSII of dark-adapted plants (**a**), F_V/F_O = The efficiency of the water-splitting complex on the donor side of PSII (**b**), F_V'/F_M' = The quantum efficiency of excitation energy trapping of PSII (**c**), ΦPSII = The quantum efficiency of PSII open centers in the light-adapted state, referred to as ΦPSII (**d**), ETR = Electron transport rate (**e**), qL = The fraction of open PSII reaction centers (**f**) and NPQ = Non-photochemical quenching (**g**). The values are presented as the mean ± standard error

2016). The dry weight of shoot was not affected in Remzibey-05 and wild safflower in treatments, whereas it was decreased under drought and increased following re-watering in Linas (about 20% decrease and increase, respectively) (Fig. 1b). Dry weight accumulation in genotypes can be connected with the accumulation of specific proteins and drought adaptability (Urban et al. 2017). The

results of dry weight which directly reflect the effects of stress on biomass showed that Remzibey-05 and C. oxyacantha have higher drought tolerance potential than Linas. In addition, the RWC of genotypes was declined under drought (more than 10%) but noticeably increased after re-watering and reached control levels, except for Linas (Fig. 1c). These results showed that Remzibey-05 and C.
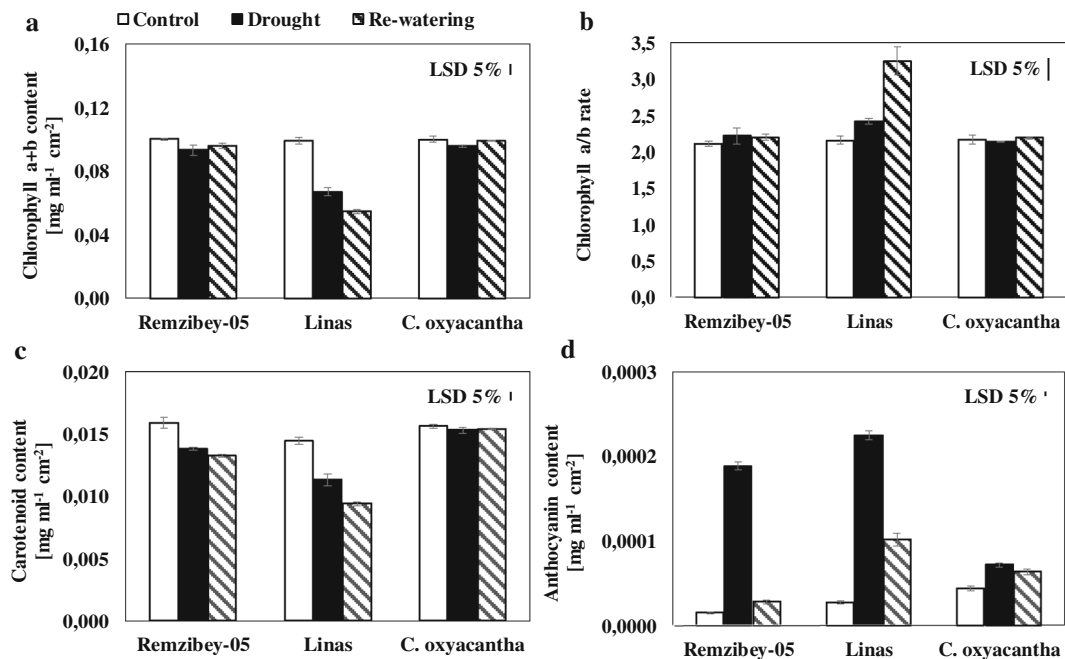
Fig. 3 Chlorophyll (a + b) = Chl a + b (**a**), chlorophyll a / b = Chl a / b ratio (**b**), carotenoid (**c**) and anthocyanin (**d**) content in the leaves of safflower genotypes exposed to drought and re-watering periods. The values are presented as the mean ± standard error

oxyacantha can rapidly revert to the previous leaf water content and development of plants during the re-irrigation period.

Many metabolic processes including photosynthesis were negatively affected under drought conditions. Our research has indicated that the photochemical activity of genotypes was affected differently from drought (Fig. 2). F_V/F_M and F_V/F_O, were decreased in Remzibey-05 and Linas under drought (Fig. 2a, b). These reductions demonstrated that water-splitting complex and reaction centers of PSII are damaged (photochemically inactive), indicating impairment of photosynthetic electron transport (Kalaji et al. 2011). These reductions induced a decrease in F_V'/F_M' for both cultivars, however to a greater extent in Linas (Fig. 2c). Besides, after re-watering, increases in F_V/F_M, F_V/F_O and F_V'/F_M' of Remzibey-05 exhibited that damage to PSII and electron transport in tolerant cultivar is reversible. The decrease in F_V'/F_M' also induced the decline in ΦPSII of cultivars, which indicates disruptions in electron transfer in response to stress (Fig. 2d). Huang et al. (2013) reported that the ΦPSII was related to F_V'/F_M', and the parameters were decreased in ramie under drought. Moreover, drought reduced ETR and q_L (Fig. 2e, f) and these reductions showed that the disruption between the excitation rate and electron transfer rate caused an increase in the reduced state of PSII reaction centers and suppression of electron transport efficiency between photosystems under drought (Efeoğlu et al. 2009). Following re-watering, ΦPSII, ETR and qL were increased in both cultivars and

these values reached the level of control in Remzibey-05. In the drought tolerant wild genotype, F_V'/F_M', ΦPSII, ETR and q_L were decreased under drought and increased to the level of control after re-watering (Fig. 2c–f). The thermal dissipation process (NPQ) of cultivars exhibited no significant change for treatments while in *C. oxyacantha*, the NPQ increased 2 to 1.72 times in drought and re-watering compared to control, respectively (Fig. 2g). The results of the study indicated that drought has a direct effect on the photosynthetic mechanisms of *C. oxyacantha* and Remzibey-05, and the tolerant genotypes were able to improve the impairments in PSII activity, electron transport and photochemical use when water was re-supplied. In parallel, photochemical parameters showed that drought disrupted PSII activity in Linas even though plant death was not observed at stressed conditions.

Drought affected not only the photochemical apparatus but also photosynthetic pigment contents. Chlorophyll (Chl a + b) content was decreased under treatments compared to control only in Linas (Fig. 3a). The reason for the decrease in Chl content of Linas could be the destruction of chlorophyll molecules while producing ROS and causing structural damage in chloroplasts (Ahmadikhah and Marufinia 2016). At the same time, the Chl a/b ratio (antenna size) was increased during the re-watering period compared to control and drought in Linas and this result indicated a decrease in antenna size after re-watering (Fig. 3b). Also, a decrease in photosynthetic activity may be partly due to decreased Chl content in Linas. However, Chl
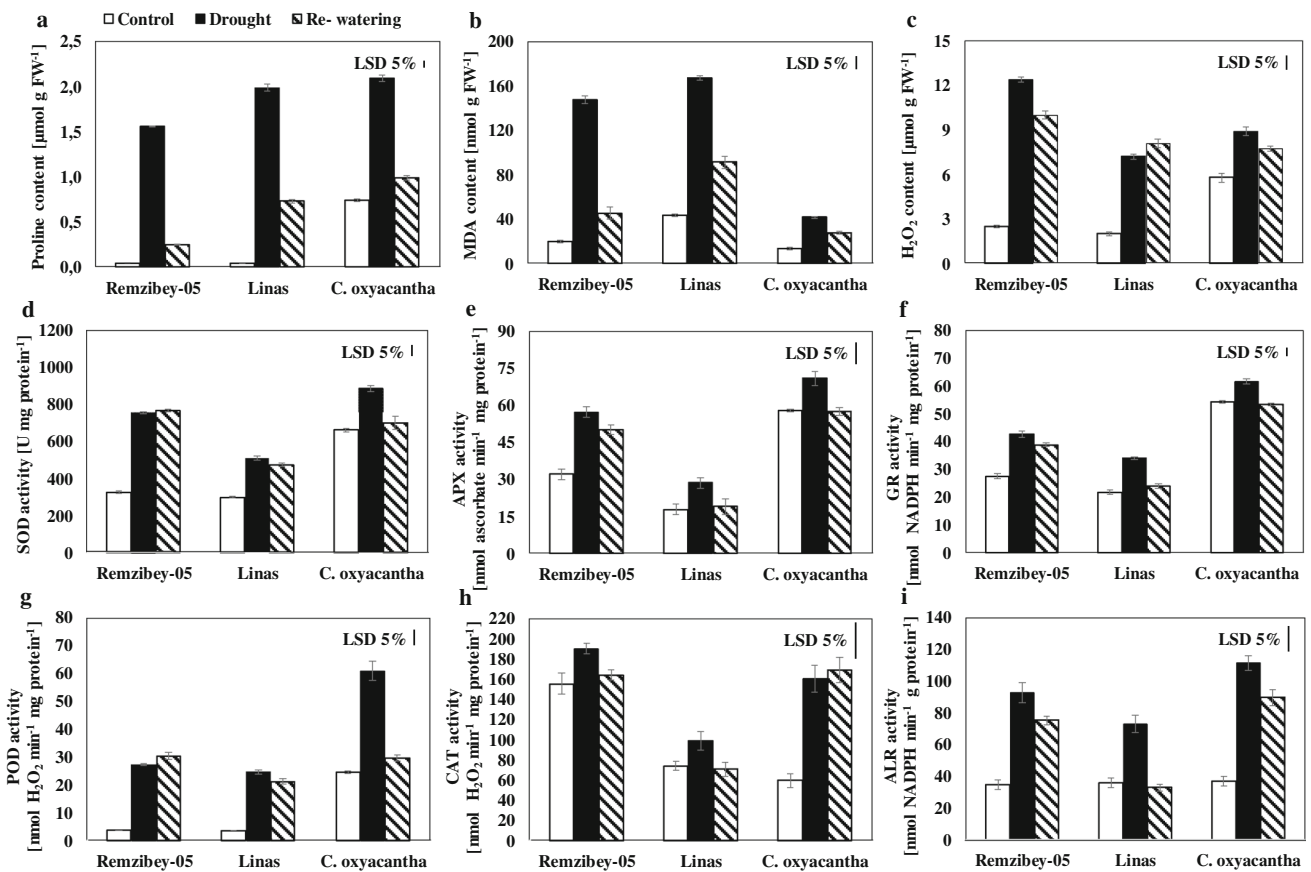
Fig. 4 Drought and re-watering periods induced changes contents of proline (**a**), MDA = malondialdehyde (**b**) and H$_2$O$_2$ = hydrogen peroxide (**c**); and the activities of SOD = Superoxide dismutase (**d**), APX = Ascorbate peroxidase (**e**), GR = Glutathione reductase (**f**), POD = Guaiacol peroxidase (**g**), CAT = Catalase (**h**) and ALR = Aldose reductase (**I**) in the leaves of safflower genotypes. The values are presented as the mean ± standard error

content and Chl a/b ratio of Remzibey-05 and *C. oxyacantha* exhibited no significant change after treatments. The carotenoid content was significantly decreased in cultivars while its content in *C. oxyacantha* remained stable throughout the treatments (Fig. 3c). On the other side, the accumulation of anthocyanin is one way of protecting against photoinhibition; by masking Chls as a ROS scavenger and filter to prevent high light absorption by leaves (Farrant 2000). Anthocyanin content was increased under treatments in all genotypes (Fig. 3d). It is possible that anthocyanin may have played an effective role in protecting Chls according to the changes in the protective pigment contents.

Osmotic stress is induced due to the reduced ability of water uptake of plants under drought. In the present study, proline which has a function to maintain osmotic potential, increased 38.00, 56.66 and 2.85-times by drought compared to control, and decreased 84%, 64% and 53% following re-watering period compared to drought for Remzibey-05, Linas and *C. oxyacantha*, respectively (Fig. 4a). Accumulation of proline in genotypes may have

been due to protein synthesis or degradation. Besides, following re-watering, the proline content of all genotypes was significantly reduced, and this decrease could be due to the use of proline as a source of nitrogen reserves during re-watering (Urban et al. 2017). Mohammadi et al. (2016) reported that a reduction in Chl content caused an increase in proline accumulation in less tolerant cultivar under drought conditions, because proline and Chl have the same precursors (synthesized from glutamate). These explanations have supported the findings of Chl and proline in Linas (Figs. 3a, 4a). Besides that, osmotic stress triggers the increase in ROS production, which causes the deterioration and peroxidation of lipid membranes in plant cells. Our results showed that MDA and H$_2$O$_2$ contents of safflower genotypes were markedly increased with drought while the values of these parameters were decreased after re-watering in Remzibey-05 and *C. oxyacantha* (Fig. 4b, c). Filippou et al. (2011) demonstrated that the content of MDA and H$_2$O$_2$ in leaves and roots of *Medicago truncatula* were increased in drought while decreased following re-watering. This recovery due to a decrease following re-

watering could be explained by presence of a rapid phospholipid renewal mechanism for the maintenance of membrane integrity and that H_2O_2 acts as a signal molecule in these genotypes.

Plants activate antioxidant defense mechanisms to scavenge ROS caused by drought. In our study, the enzyme activities of all safflower genotypes increased within a certain level to maintain the stability of ROS due to the intensity of drought (Fig. 4d–g). Following re-watering, SOD, APX, GR and POD activities in *C. oxyacantha* and APX and GR in Linas decreased while enzyme activities of Remzibey-05 did not change significantly compared to drought. Also, there were significant increases in CAT activities of Remzibey-05 and *C. oxyacantha* under drought (Fig. 4h). These results suggested that Remzibey-05 maintained a balance of the generation and elimination of ROS during treatments. Wei et al. (2020) reported that the antioxidant enzyme activity was higher in drought-tolerant safflower varieties during drought stress. However, reduction in enzyme activities of Linas did not play an active role in the removal of ROS following re-watering. Furthermore, the higher H_2O_2, proline, anthocyanin and antioxidant enzyme activities in the control of wild genotype, as well as less increment in drought compared to control cultivars and return to control level following re-watering, indicated that the *C. oxyacantha* was always prepared for unsuitable conditions and can maintain its metabolism to the optimum level under these conditions.

Drought affects the expression levels of enzymes which has a role in other metabolic functions besides defense mechanisms in plants. ALR is one of the key enzymes of the polyol metabolic pathway that catalyze the NADPH dependent reduction of the aldehyde form of glucose to sorbitol, and have an active role in the synthesis of osmotic regulators, repression of lipid peroxidation and detoxification of ROS under stress conditions as well as holding low levels of stress-induced damage and maintenance of tolerance (Oberschall et al. 2000; Tari et al. 2010). Our results showed that ALR activities of genotypes were significantly increased with drought while the level of enzyme activities was decreased following re-watering (Fig. 4i). Also, the changes in ALR enzyme activities were supported with the results of proline, lipid peroxidation and H_2O_2 in genotypes (Fig. 4a–c).

Proteomic analysis of differentially abundant proteins under drought and re-watering periods

To determine the mechanism by which safflower genotypes respond to drought and re-watering periods, 2-D PAGE was utilized to compare the leaf proteome of genotypes. Protein spots obtained from 2-D PAGE gels showed high reproducibility among the three independent extractions

based on the analysis of PDQuest software (version 8.0.1, BioRad, USA). Based on at least 2-fold quantitative change, 650 ± 30 spots were detected in all genotypes. When 2-DE maps of control and treated samples were compared, 23, 21 and 28 protein spots were identified as differentially expressed in Remzibey-05, Linas and *C. oxyacantha*, respectively (Fig. 5). These proteins were successfully identified by MALDI-TOF/TOF–MS and database searches (Uni-Prot and Swiss-Prot). The isoelectric point, molecular mass and other parameters of identified protein are shown in Supplementary Table S2 in Supplemental Materials.

To understand how DEPS are related and how the proteins involved in different pathways cross-link to each other, we constructed a protein–protein interaction (PPI) network for DEPs both for each genotype separately and all genotypes together, using the STRING database (Supplementary Fig. S1). The *Arabidopsis thaliana* was used as the reference to get homolog proteins due to unavailability of PPI data of safflower proteins in the String-database (Supplementary Table S3). In genotypes, most of the interacting proteins consisted of two major groups: photosynthesis and carbohydrate metabolism. When the genotypes were evaluated together, more protein interactions were revealed compared to individual evaluations. Ribulose bisphosphate carboxylase large chain (RBCL) is the central core protein of the PPI network due to its interactions with other proteins involved in photosynthesis and carbohydrate metabolism when genotypes evaluated together and also individually (Supplementary Fig. S1a-d). Besides, proteins involved in photosynthesis and carbohydrate metabolism interacted at high levels with each other and with proteins involved in protein (CDSP32, RPS18, RPL14, PBF1) and defense (CSD2) metabolism in genotypes. Further, the functions of DEPs in metabolic pathways are discussed.

Photosynthesis and carbohydrate metabolism

Drought has a direct impact on photosynthetic efficiency by disrupting all major components of photosynthesis and affect other primary metabolic processes such as carbohydrate metabolism (Farooq et al. 2009). In this study, 34 DEPs were associated with photosynthesis and carbohydrate metabolism after treatments (Supplementary Table S2, Fig. 7). Our proteomic analysis revealed that drought-induced changes in the expression of proteins related to photosystem reaction centers and energy transfer. Oxygen-evolving enhancer protein 2 (OEE2) which is a subunit of PSII was increased in Linas (spots 27 and 37) whereas decreased in *C. oxyacantha* (spot 55) under drought. The increase in abundance of OEE2 may be required to repair protein damage caused by dissociation
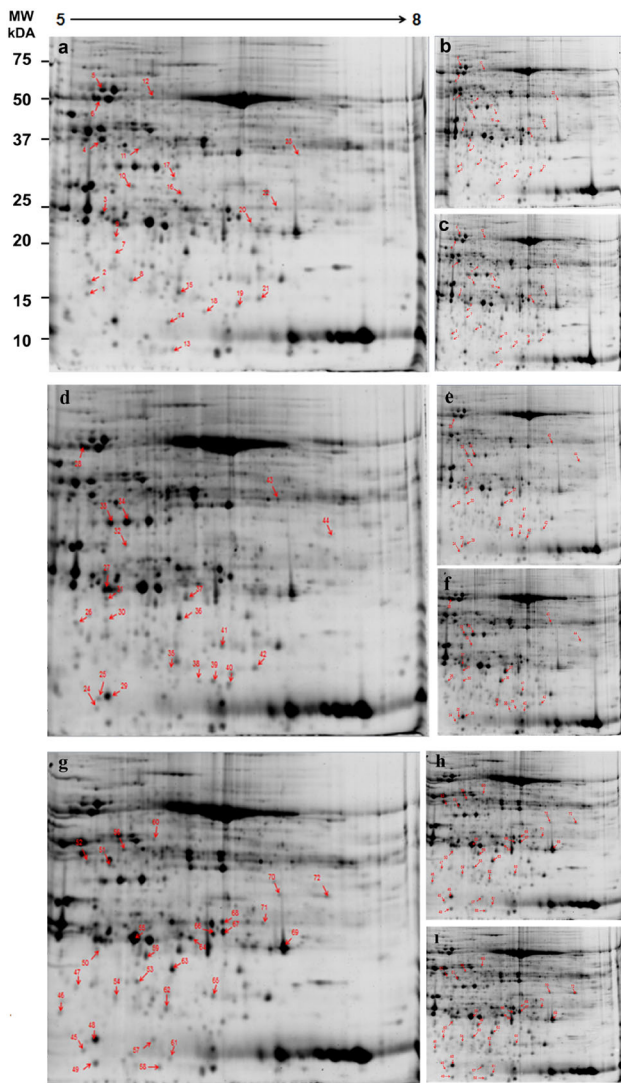
Fig. 5 2-DE gel analysis of proteins extracted from leaves of safflower genotypes during drought and re-watering periods. Representation of 2-DE protein profile for control (**a**, **d**, **g**); drought (**b**, **e**, **h**) and re-watering (**c**, **f**, **i**) treatments of Remzibey-05, Linas and *C. oxycantha*, respectively. Arrows and marked with numbers shown in red on the images of 2-D PAGE gels indicate the protein spots selected for MALDI- TOF/TOF–MS analysis

and to maintain oxygen-evolution (Gazanchian et al. 2007), thus maintaining PSII activity and protecting the mechanism of photosynthesis (Wei et al. 2020). However, this change in protein abundance may also be an indicator of its functional suppression in the PSII complex (Azri et al. 2020). After re-watering, the abundance of OEE2 was changed in different levels in Linas (downregulation in spot 27; upregulation in spot 37) while increased in *C. oxyacantha*. Besides, Cytb$_6$/f iron-sulfur subunit abundance was diminished in Linas (spot 41) and *C. oxyacantha* (spot 65) under drought, while increased in both genotypes following re-watering. Under drought, cyt b6/f were able to

prevent excessive reductions to be limited in electron transport capacity by regulating the activity of PSII (Genty and Harbinson 1996; Li and Ma 2012), and genotypes also tried to overcome the reduction in photosynthetic electron transport activity by increasing the amount of Cyt b6/f after re-watering. One of the other components of photosynthesis, Photosystem I assembly protein ycf3 was decreased in drought while increased due to subsequent re-watering in Remzibey-05 (spot 18). Photosystem I assembly protein ycf3 interacts with at least two PSI core subunits and acts as a chaperone-like factor (Nellaepalli et al. 2018). Changes in protein abundance after treatments were supported by performance index (PI$_{total}$) values, which express the use of energy from photons absorbed by PSII to reduce PSI end receptors (Çulha-Erdal et al. 2016). These results suggest that photosystem I assembly protein in Remzibey-05 may have a role in improving the negative effects of drought under re-watering periods. In addition to these, drought stress has been shown to decrease the abundance of Ferredoxin-NADP reductase (Zadražnik et al. 2013). Similar results were obtained in this study and Ferredoxin-NADP reductase, leaf isozyme 1 accumulation was decreased under drought while increased after re-watering and reached control levels in Linas (spot 34).

In this study, four subunits of ATP synthase–ATPase (alpha, beta, b and epsilon chain) showed a decrease in drought while an increase in abundance following re-watering in Remzibey-05 (spots 5, 6, 14 and 21) (Fig. 7). Also, the two isoenzymes of the ATP synthase epsilon chain described in *C. oxyacantha* (spot 48 and 57), and responded similarly to the subunits in Remzibey-05 (Fig. 7). A decrease of the ATPase activity in genotypes was demonstrated to mediate non-photochemical quenching that protects photosynthetic apparatus from photodamage under drought (Tamburino et al. 2017). However, these genotypes may provide the necessary ATP synthesis by increasing cyclic electron flow despite decrease in ATPase accumulation under stress conditions (Lv et al. 2020). An increase of the ATPase activity in genotypes following re-watering, suggests their putative role in the cellular process to meet energy demand and alleviate the effect of stress by increasing ATP supply. Besides, the accumulations of ATP synthase subunit beta (spot 28), subunit b (spot 42) and epsilon chain (spot 29) differently changed in Linas under drought and re-watering. Changes in the abundance of ATPase subunits at different levels suggested that ATP cannot be synthesized from this protein.

Changes in photosynthetic proteins were consistent with ChlF parameters of genotypes (Fig. 2 and 7). Photochemical activities decreased due to the reduction of excitation energy trapping and electron transport of photosystems (PSI and PSII) under drought, whereas the photosynthetic

efficiency of tolerant genotypes recovered with re-watering. This response could be due to the change in expression of photosynthetic proteins. But, Linas could not exhibit enough recovery with the changes in protein expressions.

Beta carbonic anhydrase-βCA (spot 22 and spot 71) which is one of the proteins that significantly affects the Calvin cycle in Remzibey-05 and *C. oxyacantha*, respectively, was diminished under drought, whereas its amount increased following re-watering. This situation in the protein amount of tolerant genotypes may indicate that βCA acts as a signal messenger under stress conditions (DiMario et al. 2017; Li et al. 2020). 10 differentially accumulated protein spots related with the large/small subunit of RuBisCO (spots 2,13, 16, 35, 36, 39, 40, 61, 63, and 68) were identified. It has been stated that RuBisCO subunits are susceptible to drought and may be caused to dissociate into subunits (Budak et al. 2013). RuBisCO large (spots 16, 2 and 63) and small (spot 13, 61 and 68) subunits were generally decreased under drought and increased following re-watering in tolerant genotypes. Under drought, the photosynthetic downregulation could be explained by a reduction in the RuBisCO subunits accumulation, and the subunit's contents could be restored following recovery (Demirevska et al. 2009). Conversely, RuBisCO large (spots 35 and 36) and small (spot 39 and 40) subunit components were increased under drought and decreased after re-watering in Linas. The increased abundance of protein fragments could indicate that RuBisCO subunits can be cleaved by ROS generated at the metal-binding site and consequently increased protein degradation rate (Hajheidari et al. 2005). Another enzyme, phosphoribulokinase (PRK) was diminished under drought, while increased following re-watering in Remzibey-05 (spot 4). It was reported that a decrease in PRK accumulation affected the Ribulose-1,5-bisphosphate (RUBP) regeneration under drought, whereas these proteins recovered following re-watering in Kentucky bluegrass cultivars (Xu et al. 2013). However, phosphoglycerate kinase (PGK) was defined in *C. oxyacantha,* and accumulation of PGK 2 (spot 60) was diminished by more than 20% under drought, while this protein and Ribulose phosphate 3 epimerase (R5P3E) (spot 66) content increased by more than 65% after re-watering in *C. oxyacantha*. These results were compatible with the changes in photochemical activity (Supplementary Table S2, Fig. 2) and indicated that the photosynthesis mechanism was maintained by repairing the damage in light reactions and Calvin cycle that is caused by drought in wild genotype. The enzymes of the glycolytic pathway, triosephosphate isomerase – TPI (spot 67), enolase (spot 12) and Putative 4-hydroxy-4-methyl-2-oxoglutarate aldolase 1 (spot 8), exhibited similar responses with enzymes of Calvin cycle in tolerant genotypes. The reduction in protein tolerant genotypes might indicate that the transport of photoassimilates from source to sink tissues were restricted, when photosynthesis was diminished due to drought. Urban et al. (2017) reported that the changes in the proteins involved in carbohydrate metabolism were compatible with a decrease of photosynthesis-related proteins in rapeseeds. Also, an increase in the abundance of these enzymes following re-watering could be related to a need for extra energy to repair damage caused by stress.

Protein metabolism

The second largest group of protein metabolism with different accumulation in treatments was composed of proteins involved in protein synthesis (transcription and translation), storage and proteolysis. The accumulation of Cysteine synthase-CS (spot 11) which catalyzes the final step of the cysteine biosynthesis was decreased by 74% in drought while increased about 3 times following re-watering when compared to stress in Remzibey-05. The decrease in cysteine biosynthesis could support a reduction in sulphur absorption and formation of glutathione, methionine, and sulphurated secondary metabolites under drought (Tamburino et al. 2017). Another enzyme, Glutamine synthetase-GS (spot 56) was decreased under treatments in *C. oxyacantha*. This decrease may indicate a disruption in nitrogen assimilation that leads to the formation of organic nitrogen compounds like amino acids (Valero-Galván et al. 2013). Chloroplast stem-loop binding protein 41 kDa a-CSP41a (spot 43), associated with transcription and translation was increased under drought and decreased following re-watering in Linas. The change in the accumulation of CSP41a protein in Linas supported the changes in the accumulation of proteins encoded in chloroplast and involved in photosynthesis under treatments. As seen in the change of the proteins involved in photosynthesis and carbon metabolism in treatments, chloroplastic ribosomal proteins (spot 4, 9 and 58) were diminished in Remzibey-05 and *C. oxyacantha*, respectively, whereas chloroplastic ribosomal protein (spot 24) was increased in Linas under drought, and these proteins were generally increased following re-watering in genotypes. It has been reported that the accumulation of ribosomal proteins was changed in response to drought stress (Zadražnik et al. 2017; Nemati et al. 2019). In our study, changes in the amount of ribosomal proteins in genotypes support the changes in the accumulation of proteins which is encoded by the chloroplast DNA as subunits of PSI, PSII, ATPsynhase and Cyt-b6f complex and RuBisCO large subunit (Supplementary Table S2, Fig. 7). Besides, mitochondrial ribosomal protein S31 (spot 33) was decreased in treated plants in Linas. In addition to these proteins, Phaseolin alpha-type (spot 7) was increased about 3.8 times under drought whereas decreased by 53%

following re-watering in Remzibey-05. It was demonstrated that some proteins may be stored in the vacuole for use as needed, rather than degradation under stress (Badowiec and Weidner 2014). Furthermore, these proteins may have been used to produce the required energy and /or to repair any damage after re-watering in Remzibey-05.

One of the proteolysis-related proteins, proteasome subunit beta type-1 (spot 20) was increased more than 2-fold under drought and following re-watering compared to control in Remzibey-05. Proteolysis-related proteins, which are necessary for the maintenance of cellular protein homeostasis, break down the proteins damaged by stress (Zadražnik et al. 2013). The accumulation of proteasome may point out to have key role in degradation of incorrectly folded, damaged or dysfunctional proteins in the tolerant cultivar. However, the accumulation of the proteasome subunit beta type-3-B (spot 31) was reduced under drought and following re-watering in Linas, and this decrease may indicate that the damaged or dysfunctional protein cannot be sufficiently proteolyzed under both conditions in this genotype.

Defense metabolism

Several DEPs that have a role in ROS detoxification and chaperone activity, have also been observed to have a role in the defense responses. The abundance of chloroplastic superoxide dismutase [Cu–Zn] (spot 15) and peroxidase 43 (spot 1) was decreased under drought and following re-watering in Remzibey-05 whereas the accumulation of SOD (spot 62) was only increased following re-watering in *C. oxyacantha.* Although total SOD and POD activity in the genotypes were increased after treatments, the response of these proteins may indicate that other SOD isoenzymes (MnSOD–FeSOD) and/or cellular forms (cytosolic and mitochondrial Cu/ZnSOD) have more effective function than chloroplastic superoxide dismutase and peroxidase 43 (Fig. 4d–g). Apart from these proteins, chloroplastic thioredoxin-like protein CDSP32 (spot 32) and Glycine-rich RNA-binding protein (spot 25) was decreased under drought, while increased following re-watering in Linas. Thioredoxins like (TRX-like) proteins have a role in controlling the function and structure of proteins by reducing disulfide bridges in the redox-active site (Nikkanen et al. 2014) while Glycine-rich RNA binding protein has RNA chaperone activity to protect from stress (Kim et al. 2007). The decrease in accumulation of both proteins showed that these protection systems were not functioning adequately under drought. However, the genotypes attempted to improve the damages occurring in the chloroplast, and perform biological functions of RNA by completing the processes of folding and/or assembling correctly following re-watering.

Energy metabolism

Putative cytochrome c oxidase subunit II PS17 was the only identified protein related to energy metabolism, and it was detected as multiple spots on the 2-D gel of genotypes (spots 10, 23, 26, 30, 45–47, 49, 50, 52–54, 59, 64 and 69). These variations may be caused by various factors such as protein isoforms, comigrations, degradation and partial synthesis, and protein translation from alternatively spliced mRNAs, or errors in the database sequence (partial protein sequence) due to post-translational modifications (Valero-Galván et al. 2013). The abundance of putative cytochrome c oxidase subunit II PS17 was changed in Linas after treatments (spots 26 and 30) while it was decreased under drought and increased following re-watering in Remzibey-05 (spots 10 and 23) and *C. oxyacantha* (spots 45–69). It has been reported that a putative cytochrome c oxidase subunit II PS17 was detected in multiple spots and decreased in wild type wheat under drought (Budak et al. 2013). Changes in the amount of cytochrome c oxidase were accompanied by changes in the amount of protein in photosynthesis and carbohydrate metabolism in genotypes after treatments and these results revealed the level of interaction between chloroplast and mitochondria, with the energy and metabolic connection between the two organelles and cytosol.

Other proteins involved in drought

Xyloglucan endotransglucosylase/hydrolase protein (spot 70), was increased under drought and did not change significantly following re-watering compared to drought in *C. oxyacantha.* This increase may contribute to cell wall remodeling to strengthen the wall layers and protect mesophyll cells from stress (Ye et al. 2016). Among the proteins involved in the MAP signaling pathway, Rac-like GTP-binding protein 2-RAC (spot 38) decreased and increased under drought and re-watering, respectively in Linas, whereas Serine/threonine protein phosphatase 2A – PP2A (spot 20) was accumulated more than 1.5-times in both treatments in *C. oxyacantha*. RAC-like proteins and protein phosphatases are involved in plant defense response to biotic/abiotic stresses as well as plant growth and development (Haider et al. 2017; Yang et al. 2018). The changes in the accumulation of a RAC-like protein indicated that cellular functions were adversely affected under drought in Linas and the genotype tried to overcome the damage with re-irrigation, whereas the accumulation of PP2A may be an indication of resistance level in the wild type genotype. Another protein, Chloroplastic NDPK II (spot 19) was diminished about by 44% under drought while increased by 53% following re-watering in Remzibey-05. Alteration in the amount of NDPK was consistent

with changes in the accumulation of proteins involved in photosynthesis and protein metabolism in the chloroplast (Supplementary Table S2, Fig. 7). Besides these proteins, Gibberellin 3-beta-dioxygenase 2–1 (spot 17) was decreased under drought and was not changed following re-watering in Remzibey-05. Decreasing GA levels may have caused a limitation in plant growth under drought (Cole-brook et al. 2014). Apart from these proteins, Germin-like protein 5–1 (GLPs 5–1) (spot 44) and Putative LRR disease resistance protein/transmembrane receptor kinase PS16 (spot 72) were increased under drought and decreased following re-watering in Linas and *C. oxyacantha,* respectively. Although, their exact role in metabolism is not fully understood, it is thought that these proteins are associated with biotic and abiotic stress resistance (Smith et al. 2006; Urban et al. 2017). Detailed explanation of the relationship between these proteins and drought may contribute in deciphering their exact functions.

### Validation of selected DEP expression by RT-qPCR

Quantification of mRNA levels of three selected proteins, ATP synthase subunit beta chain (defined in Remzibey-05 and Linas), RuBisCO large chain (co-defined in genotypes) and photosystem I binding protein ycf (defined in Remz-ibey-05) were performed by RT-qPCR (Fig. 6). These results confirmed the correlation between the expression changes of RNA and the level of corresponding proteins (Supplementary Table S2, Fig. 6). The expression level of ATP synthase subunit beta chain and photosystem I

binding protein ycf in Remzibey-05, and RuBisCO large chain in Remzibey-05 and *C. oxyacantha* were decreased under drought while increased following re-watering. However, the transcript level of ATP synthase subunit beta protein was observed as increased in treated plants as in the change of protein accumulation in Linas (spot 28). A dis-crepancy was seen between mRNA and protein abundances (spots 35 and 36) of RuBisCO large chain in Linas. The incongruence between transcript level and corresponding protein abundance of RuBisCO large chain may be asso-ciated with a time delay between mRNA production and translation of the related proteins, and possible turnover. Also the rates of translation and protein degradation differ among different genes and proteins, and post-transcrip-tional and translational regulatory mechanisms (Xin et al. 2018).

Conclusion

Drought is one of the abiotic stress factors that causes significant yield losses in crops, particularly oil crops. The present study showed that fresh and dry weights of geno-types were affected by drought, but this effect was observed to be more drastic in Linas. It has been observed that wild *C. oxyacantha* was more successful in drought tolerance than cultivars via activating the photochemical efficiency and defense systems, as well as protecting membrane stability and water status. Protein profiles of leaf in safflower genotypes showed that photosynthesis and

Fig. 6 RT-qPCR of selected proteins in safflower genotypes exposed to drought and re-watering periods. The values are presented as the mean ± standard error
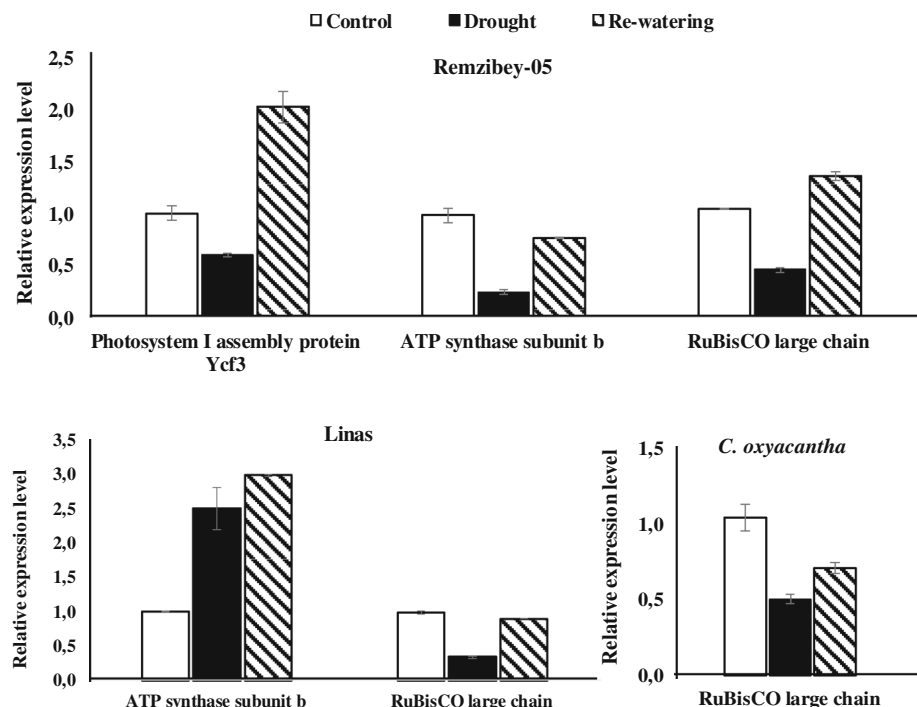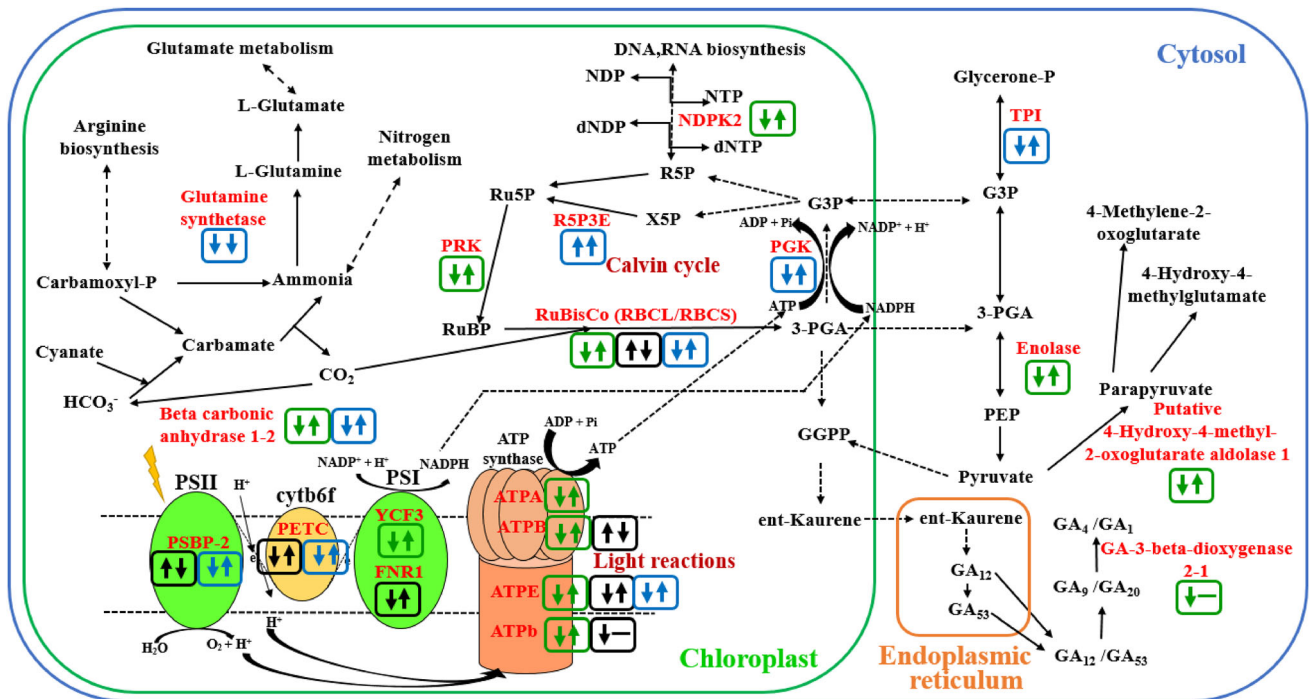
Fig. 7 The effect of drought and re-watering periods on the photosynthetic machinery, Calvin cycle, glycolysis, gluconeogenesis and nitrogen, purine, pyrimidine and hormone metabolism. The scheme was drawn by modifying KEGG metabolic pathways. Also, green, black and blue squares represent changes in Remzibey-05; Linas and *C. oxyacantha*, respectively. Besides, the first and second arrows indicate change according to control in drought and to drought in re-watering, respectively. The down and up arrow indicate a decrease and an increase of protein abundances, respectively. When a protein or subunit is defined in several spots in genotypes, the direction of the arrows is determined to average change levels

carbohydrate metabolism were the most affected metabolic pathways although multiple metabolic processes altered during treatments. Furthermore, Remzibey-05 tolerant cultivar repaired the stress-induced damage by increasing the expression of the proteins involved in photosynthesis and carbohydrate metabolism during recovery, as in wild *C. oxyacantha*. Chlorophyll a fluorescence characteristics supported the changes seen in protein profiles related to the photosynthetic process in genotypes. Our study provides insight into the underlying response mechanism to drought tolerance in safflower genotypes which may be of value for future studies investigating drought responses of oilseed plant species. Thus, we suggest that Remzibey-05 could be grown in areas where drought is a threat and used as a parent in the safflower breeding program, together with the wild type genotype.

Author contributions Conceived and designed the experiments: ŞÇE, FE and YE. Performed the experiments: ŞÇE. Analyzed the data: ŞÇE and YE. Wrote the paper: ŞÇE and YE All authors gave final approval of the submitted and published versions.

Compliance with ethical standards

Conflict interest The authors declare that they have no conflicts of interest.

References

Ahmadikhah A, Marufinia A (2016) Effect of reduced plant height on drought tolerance in rice. 3 Biotech 6:221. https://doi.org/10.1007/s13205-016-0542-3

Ahsan N, Lee D-G, Alam I et al (2008) Comparative proteomic study of arsenic-induced differentially expressed proteins in rice roots reveals glutathione plays a central role during as stress. Proteomics 8:3561–3576. https://doi.org/10.1002/pmic.200701189

Azri W, Cosette P, Guillou C et al (2020) 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

Badowiec A, Weidner S (2014) Proteomic changes in the roots of Germinating *Phaseolus vulgaris* seeds in response to chilling stress and post-stress recovery. J Plant Physiol 171:389–398. https://doi.org/10.1016/j.jplph.2013.10.020

Bates L, Waldren R, Teare I (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207. https://doi.org/10.1007/BF00018060

Bergmeyer HU (1974) Methods for determination of enzyme activity. In: Bergmeyer HU (Ed) Methods of Enzymatic Analysis, Vol II, UK:Academic Press, London, pp 685–690.

Beyer WF, Fridovich I (1987) Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem 161:559–566. https://doi.org/10.1016/0003-2697(87)90489-1

Bilger W, Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in Hedera canariensis. Photosynth Res 25:173–185. https://doi.org/10.1007/BF00033159

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Budak H, Akpinar BA, Unver T et al (2013) 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

Budak H, Hussain B, Khan Z et al (2015) From genetics to functional genomics: ımprovement in drought signaling and tolerance in wheat. Front Plant Sci 6:1012. https://doi.org/10.3389/fpls.2015.01012

Chance B, Maehly, (1955) A Assay of catalases and peroxidases. Methods Enzymol 2:764–775. https://doi.org/10.1002/9780470110171.ch14

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159. https://doi.org/10.1006/abio.1987.9999

Claeys H, Inzé D (2013) The agony of choice: how plants balance growth and survival under water-limiting conditions. Plant Physiol 162:1768–1779. https://doi.org/10.1104/pp.113.220921

Colebrook EH, Thomas SG, Phillips AL et al (2014) The role of gibberellin signalling in plant responses to abiotic stress. J Exp Biol 217:67–75. https://doi.org/10.1242/jeb.089938

Çulha-Erdal Ş, Nalcaiyi ASB, Eyidoğan F et al (2016) Probing the responses of safflower genotypes (Carthamus tinctorius L.) under drought stresses and re-watering using performance indexes. Plant Biology Europe EPSO/FESPB 2016 Congress.

Demirevska K, Zasheva D, Dimitrov R et al (2009) Drought stress effects on Rubisco in wheat: changes in the Rubisco large subunit. Acta Physiol Plan 31:1129–1138. https://doi.org/10.1007/s11738-009-0331-2

DiMario RJ, Clayton H, Mukherjee A et al (2017) Plant carbonic anhydrases: structures, locations, evolution, and physiological roles. Mol Plant 10:30–46. https://doi.org/10.1016/j.molp.2016.09.001

Efeoğlu B, Ekmekçi Y, Çiçek N (2009) Physiological responses of three maize cultivars to drought stress and recovery. S Afr J Bot 75:34–42. https://doi.org/10.1016/j.sajb.2008.06.005

Esterbauer H, Cheeseman KH (1990) Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol 186:407–421. https://doi.org/10.1016/0076-6879(90)86134-H

Farooq M, Wahid A, Kobayashi N et al (2009) Plant drought stress: effects, mechanisms and management. Agron Sustain Dev 29:185–212. https://doi.org/10.1051/agro:2008021

Farrant JMA (2000) Comparison of mechanisms of desiccation tolerance among three angiosperm resurrection plant species. Plant Ecol 151:29–39. https://doi.org/10.1023/A:1026534305831

Filippou P, Antoniou C, Fotopoulos V (2011) Effect of drought and rewatering on the cellular status and antioxidant response of Medicago truncatula plants. Plant Signal Behav 6:270–277. https://doi.org/10.4161/psb.6.2.14633

Gazanchian A, Hajheidari M, Sima NK et al (2007) Proteome response of Elymus elongatum to severe water stress and recovery. J Exp Bot 58:291–300. https://doi.org/10.1093/jxb/erl226

Gecgel U, Demirci M, Esendal E et al (2007) Fatty Acid composition of the oil from developing seeds of different varieties of safflower (Carthamus tinctorius L.). J Am Oil Chem Soc 84:47–54. https://doi.org/10.1007/s11746-006-1007-3

Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta (BBA)-Gen Sub 990:87–92. https://doi.org/10.1016/S0304-4165(89)80016-9

Genty B, Harbinson J (1996) Regulation of light utilization for photosynthetic electron transport. In: Baker ND (ed) Photosynthesis and the Environment. Springer, Netherlands, pp 67–99

Hajheidari M, Abdollahian-Noghabi M, Askari H et al (2005) Proteome analysis of sugar beet leaves under drought stress. Proteomics 5:950–960. https://doi.org/10.1002/pmic.200401101

Haider MS, Kurjogi MM, Khalil-Ur-Rehman M et al (2017) Grapevine immune signaling network in response to drought stress as revealed by transcriptomic analysis. Plant Physiol Biochem 121:187–195. https://doi.org/10.1016/j.plaphy.2017.10.026

Huang C, Zhao S, Wang L et al (2013) Alteration in chlorophyll fluorescence, lipid peroxidation and antioxidant enzymes activities in hybrid ramie (Boehmeria Nivea L.) under drought stress. Aust J Crop Sci 7:594–599

Hurkman WJ, Tanaka CK (1986) Solubilization of plant membrane proteins for analysis by two-dimensional gel electrophoresis. Plant Physiol 81:802–806. https://doi.org/10.1104/pp.81.3.802

IPCC (2019) Global warming of 1.5°C, An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty https://www.ipcc.ch/site/assets/uploads/sites/2/2019/06/SR15_Full_Report_High_Res.pdf

Istanbulluoglu A, Gocmen E, Gezer E et al (2009) Effects of water stress at different development stages on yield and water productivity of winter and summer safflower (Carthamus tinctorius L.). Agric Water Manag 96:1429–1434. https://doi.org/10.1016/j.agwat.2009.04.004

Kalaji HM, Bosa K, Kościelniak J et al (2011) Effects of salt stress on photosystem II efficiency and CO_2 assimilation of two syrian barley landraces. Environ Exp Bot 73:64–72. https://doi.org/10.1016/j.envexpbot.2010.10.009

Kim ST, Cho KS, Jang YS et al (2001) Two-dimensional electrophoretic analysis of riceproteins by poly-ethylene glycol fractionation for protein arrays. Electrophoresis 22:2103–2109. https://doi.org/10.1002/1522-2683(200106)22:10%3c2103::AID-ELPS2103%3e3.0.CO;2-W

Kim JS, Park SJ, Kwak KJ et al (2007) Cold shock domain proteins and glycine-rich RNA-binding proteins from Arabidopsis thaliana can promote the cold adaptation process in Escherichia coli. Nucleic Acids Res 35:506–516. https://doi.org/10.1093/nar/gkl1076

Kramer DM, Johnson G, Kiirats O et al (2004) New fluorescence parameters for the determination of Q_A redox state and excitation energy. Photosynth Res 79:209–218. https://doi.org/10.1023/B:PRES.0000015391.99477.0d

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685. https://doi.org/10.1038/227680a0

Li P, Ma F (2012) Different effects of light irradiation on the photosynthetic electron transport chain during apple tree leaf dehydration. Plant Physiol Biochem 55:16–22. https://doi.org/10.1016/j.plaphy.2012.03.007

Li P, Liu H, Yang H et al (2020) Translocation of drought-responsive proteins from the chloroplasts. Cells 9:259. https://doi.org/10.3390/cells9010259

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:350–382. https://doi.org/10.1016/0076-6879(87)48036-1

Lv Y, Li Y, Liu X et al (2020) Photochemistry and proteomics of ginger (Zingiber officinale Roscoe) under drought and shading. Plant Physiol Biochem 151:188–196. https://doi.org/10.1016/j.plaphy.2020.03.021

Majidi MM, Tavakoli V, Mirlohi A et al (2011) Wild safflower species ('Carthamus oxyacanthus' Bieb.): a possible source of drought tolerance for arid environments. Aust J Crop Sci 5:1055–1063

Mancinelli AL, Yang C-PH, Lindquist P et al (1975) Photocontrol of anthocyanin synthesis: III. The action of streptomycin on the synthesis of chlorophyll and anthocyanin. Plant Physiol 55:251–257. https://doi.org/10.1104/pp.55.2.251

Mihaela P, Josef R, Monica N et al (2013) Perspectives of Safflower oil as biodiesel source for south eastern europe (comparative study: safflower, soybean and rapeseed). Fuel 111:114–119. https://doi.org/10.1016/j.fuel.2013.04.012

Mohammadi M, Ghassemi-Golezani K, Zehtab-Salmasi S et al (2016) Assessment of some physiological traits in spring safflower (Carthamus tinctorius L.) cultivars under water stress. Int J Life Sci 10:58–64. https://doi.org/10.3126/ijls.v10i1.14512

Mundree SG, Thomson WA, JA, et al (2000) An aldose reductase homolog from the resurrection plant Xerophyta viscosa Baker. Planta 211:693–700. https://doi.org/10.1007/s004250000331

Nellaepalli S, Ozawa S-I, Kuroda H et al (2018) The photosystem I assembly apparatus consisting of Ycf3–Y3IP1 and Ycf4 modules. Nat Commun 9:2439. https://doi.org/10.1038/s41467-018-04823-3

Nemati M, Piro A, Norouzi M et al (2019) Comparative physiological and leaf proteomic analyses revealed the tolerant and sensitive traits to drought stress in two wheat parental lines and their F6 progenie. Environ Exp Bot 158:223–237. https://doi.org/10.1016/j.envexpbot.2018.10.024

Nikkanen L, Rintamäki E (2014) Thioredoxin-dependent regulatory networks in chloroplasts under fluctuating light conditions. Philos T R Soc B 369:1–7. https://doi.org/10.1098/rstb.2013.0224

Oberschall A, Deák M, Török K et al (2000) A novel aldose/aldehyde reductase protects transgenic plants against lipid peroxidation under chemical and drought stresses. Plant J 24:437–446. https://doi.org/10.1046/j.1365-313x.2000.00885.x

Ramagli LS, Rodriguez LV (1985) 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

Rao MV, Hale BA, Ormrod DP (1995) Amelioration of ozone-induced oxidative damage in wheat plants grown under high carbon dioxide (role of antioxidant enzymes). Plant Physiol 109:421–432. https://doi.org/10.1104/pp.109.2.421

Robert GA, Rajasekar M, Manivannan P (2016) Triazole-induced drought stress amelioration on growth, yield, and pigments composition of Helianthus annuus L. (Sunflower). Int Multidiscip Res J 5:6–15

Smith JA, Blanchette RA, Burnes TA et al (2006) Proteomic comparison of needles from blister rust-resistant and susceptible Pinus strobus seedlings reveals upregulation of putative disease resistance proteins. Mol Plant Microbe Interact 19:150–160. https://doi.org/10.1094/MPMI-19-0150

Tamburino R, Vitale M, Ruggiero A et al (2017) Chloroplast proteome response to drought stress and recovery in tomato (Solanum lycopersicum L.). BMC Plant Biol 17:1–14. https://doi.org/10.1186/s12870-017-0971-0

Taiz I, Zaiger E, Møller IM et al (2015) Plant Physiology and Development. Sinauer Associates Publishers, Sunderland

Tari I, Kiss G, Deer AK et al (2010) Salicylic acid increased aldose reductase activity and sorbitol accumulation in tomato plants under salt stress. Biol Plant 54:677–683. https://doi.org/10.1007/s10535-010-0120-1

Urban MO, Vašek J, Klíma M et al (2017) Proteomic and physiological approach reveals drought-induced changes in rapeseeds: water-saver and water-spender strategy. J Proteomics 152:188–205. https://doi.org/10.1016/j.jprot.2016.11.004

Valero-Galván J, González-Fernández R, Navarro-Cerrillo RM et al (2013) Physiological and proteomic analyses of drought stress response in holm oak provenances. J Proteome Res 12:5110–5123. https://doi.org/10.1021/pr400591n

Wang SY, Jiao HJ, Faust M (1991) Changes in ascorbate, glutathione, and related enzyme activities during thidiazuron-induced bud break of apple. Physiol Plant 82:231–236. https://doi.org/10.1111/j.1399-3054.1991.tb00086.x

Wang Y, Xu C, Zhang B et al (2017) Physiological and proteomic analysis of rice (Oryza sativa L.) in flag leaf during flowering stage and milk stage under drought stress. Plant Growth Regul 82:201–218. https://doi.org/10.1007/s10725-017-0252-9

Wei B, Hou K, Zhang H et al (2020) Integrating transcriptomics and metabolomics to studies key metabolism, pathways and candidate genes associated with drought-tolerance in Carthamus tinctorius L. under drought stress. Ind Crop Prod 151:112465. https://doi.org/10.1016/j.indcrop.2020.112465

Xin L, Zheng H, Yang Z et al (2018) Physiological and proteomic analysis of maize seedling response to water deficiency stress. J Plant Physiol 228:9–38. https://doi.org/10.1016/j.jplph.2018.05.005

Xu L, Yu J, Han L et al (2013) Photosynthetic enzyme activities and gene expression associated with drought tolerance and post-drought recovery in kentucky bluegrass. Environ Exp Bot 89:28–35. https://doi.org/10.1016/j.envexpbot.2012.12.001

Yang Z, Liu J, Luo L (2018) The Cotton GhRac6 Gene Encoding a Plant ROP/RAC Protein Improves the plant defense response to aphid feeding. Plant Mol Biol Rep 36:888–896. https://doi.org/10.1007/s11105-018-1127-6

Ye Z, Sangireddy S, Okekeogbu I et al (2016) Drought-induced leaf proteome changes in switchgrass seedlings. Int J Mol Sci 17:1–18. https://doi.org/10.3390/ijms17081251

Zadražnik T, Hollung K, Egge-Jacobsen W et al (2013) Differential proteomic analysis of drought stress response in leaves of common bean (Phaseolus vulgaris L.). J Proteom 78:254–272. https://doi.org/10.1016/j.jprot.2012.09.021

Zadražnik T, Egge-Jacobsen W, Vladimir Meglič V et al (2017) Proteomic analysis of common bean stem under drought stress using in-gel stable isotope labeling. J Plant Physiol 209:42–50. https://doi.org/10.1016/j.jplph.2016.10.015