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ARTICLE

Proteomic Profiling and Protein-Protein Interaction Network Reveal the Molecular Mechanisms of Susceptibility to Drought Stress in Canola (*Brassica napus* L.)

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ABSTRACT

Drought stress is one of the most important abiotic stresses that plants face frequently in nature. Under drought conditions, many morphological, physiological, and molecular aspects of plants are changed and as a result plants experience a remarkable reduction in growth, yield, and reproduction. To expand our understanding of the molecular basis of the plant response to drought stress, the proteomic profile and protein-protein network of canola (*Brassica napus* L.) were studied. The focus was to show molecular mechanisms related to canola susceptibility to drought stress. The experiment used a completely randomized design, implemented in a hydroponic system under greenhouse conditions. To impose drought stress, plants were exposed to Hoagland's solution supplemented with polyethylene glycol (PEG) 6000 for 7 days. The drought stress resulted in 161reproducible protein spots in two-dimensional electrophoresis of canola leaves. The t-student test showed 21 differentially abundant proteins (DAP), of which 2 and 19 were up and down accumulated, respectively. Two spots identified as 1-aminocyclopropane-1-carboxylate oxidase and D-2-hydroxyglutarate dehydrogenase showed an increased abundance of 2.11 and 1.77, respectively. The extended protein-protein interaction of differentially abundant proteins and KEGG analysis showed 47 pathways directly and indirectly associated with canola response to drought stress. DAPs with increased abundance were associated with amino acid and signaling processes, whereas DAPs with decreased abundance were mostly connected with pathways responsible for energy production. The results of the study will help to elucidate further the molecular events associated with the susceptibility to drought stress in canola.

KEYWORDS

Abiotic stress; differentially abundant protein; molecular function; proteomics; susceptibility

1 Introduction

The challenges of abiotic stress have widely been under attention as one of the major constraints to crop production. On the other hand, increasing human population and demand for more food exacerbate these challenges alongside other constraints such as climate change and biotic stress [1]. These challenges to secure food production for the world demand crops which are resilient to such conditions [1]. Genetic engineering of crops to make them more tolerant to unfavorable conditions has been considered as one of the strategies to secure food production around the world [2]. However, a complexity of mechanisms

associated with response to abiotic stresses challenges the attempts to create tolerant cultivars [2]. This is on one hand because of the complexity of plant responses to abiotic stresses, and on the other hand because of lack of comprehensive information about the molecular basis of plant response [3].

One of the main goals of plant biology is to decipher mechanisms by which plants respond to their environment at the molecular and cellular level. In nature, abiotic stresses, especially drought, limit plant growth and distribution [4]. In addition, reduced water uptake by plants is the major constraint of other abiotic stresses such as salinity. Therefore, knowing the basics of drought response of plants could give more insight on other abiotic stresses. Many studies on different species have indicated that drought stress changes a plethora of gene expression and protein abundance (e.g., gene/proteins responsible for ion transport, carbon metabolism, synthesis of compatible solutes, and antioxidant defense systems [5–7]). Overall, the plant response could be divided into three kinds: adjusting hemostasis, detoxification of harmful elements, and recovery of growth [4].

Plants, as sessile organisms, have evolved many adaptive mechanisms at the molecular, cellular, and whole plant levels. For example, plants change the expression of their numerous genes involved in adjusting ion transport or upregulation of genes involved in the biosynthesis of protective molecules such as proline, chaperones, etc. [4,8]. Generally speaking, plants use two major strategies including avoidance in which plants survive stressful conditions in a physiologically inactive stage, and acclimation in which plants change their physiological and biochemical traits remarkably through altering numerous gene expression [9]. It has already been demonstrated that changes in gene expression do not necessarily lead to changes in the abundance of proteins [9,10]. Moreover, proteins, unlike genes, are direct effectors of plant response to internal and external stimuli [11]. Therefore, the study of plant response to its environment at the proteome level is essential to better understand the mechanisms of plant-stress interactions.

Proteomic approaches using two-dimensional gel electrophoresis (2-DE) are based on a comparison of a set of protein spots between non-stressed (control) and stressed plants. These studies have enriched abiotic stress research by providing integrated information on the alteration of the proteome profile during stress conditions [12]. Advances in bioinformatics have provided tools to handle a large set of proteomic data and improve the knowledge gain about the underlying mechanisms [13]. These studies have indicated that depending on the plant species, severity of stress, plant growth stage, and type of tissue, the response to stress varies dynamically [4,8,11]. The functional analysis of a set of DE proteins put mainly them all into one or several groups depending on carbohydrate and energy metabolism, photosynthesis, ion hemostasis, membrane trafficking, reactive oxygen species scavenging, and cytoskeleton reorganization [8]. In addition, the proportion of proteins grouped in these functional groups differ between species. For example, *Arabidopsis thaliana* alter mostly its photosynthesis-related proteins, while halophytes change their metabolism/defense-related proteins in response to the abiotic stress [8,14]. Moreover, proteomic researches have revealed that plant response at the proteome level to its surrounding environment changes even under relatively low different conditions [3]. Therefore, achieving a comprehensive understanding of the dynamic behavior of the plant proteome demands species-specific studies integrated with well-designed experiments.

Canola (*Brassica napus* L.) is one of the most important crops cultivated around the world for its valuable oil and production of biodiesel fuel [8]. Studies have indicated that canola cultivars show different degrees of tolerance to abiotic stress including drought stress [8,15,16]. Mohammadi et al. [17] reported that in a drought-sensitive cultivar of canola, tubulin beta-2 and heat shock protein 70 are significantly downregulated. Gharelo et al. [18] indicated that tolerant and sensitive cultivars of canola have different molecular mechanisms under abiotic stress. They reported that proteins involved in response to stress and antioxidant activity are lower in sensitive than tolerant cultivars. It has been demonstrated that under stressful conditions proteins related to photosynthesis, protein synthesis, and stress and defense are increased in canola [19]. There are many other proteomic studies on tolerant and

sensitive cultivars in *B. napus* [11,17–20]; however, many aspects have been studied less often on sensitive than tolerant cultivars.

To obtain a greater insight into the molecular mechanisms associated with canola susceptibility in response to drought stress, we used a susceptible cultivar of canola (*Brassica napus* L. cultivar Sarigol) that we previously proved [21,22] to study proteome changes and differentially abundant proteins (DAPs). The results of this research support most of the previous findings and provide evidence for the susceptibility of the canola cultivar to drought stress.

2 Materials and Methods

2.1 Plant Material and Cultivation

Leaf samples of canola (*Brassica napus* L. cultivar Sarigol), assigned as a salt-sensitive cultivar of canola [21,22], were used in this study. The experiment was designed based on a completely randomized design (CRD) and conducted in the research greenhouse of the Faculty of Agriculture located at the University of Tabriz. Simply, two groups of plants divided into control and drought-stressed group. The Seed and Plant Improvement Institute (Karaj, Iran) provided the seeds (*B. napus L*. cultivar Sarigol). Seeds were germinated under aseptic conditions and after one-week, seedlings were sterilized and transferred to a hydroponic system with a sterilized and modified Hoagland's solution (pH 6.5 ± 0.5 adjusted using hydrochloric acid/potassium hydroxide) [22]. Greenhouse conditions were maintained as follows during the experiment period: [temperature 25 ± 2°C, relative humidity 50% to 60% (during day and night), and 14 h period of light]. To simulate drought stress, 28-day-old plants were exposed to Hoagland's solution supplemented with polyethylene glycol (PEG) 6000 for 7 days (0.6 MPa per 10 L of nutrient solution). To make sure that plants experienced severe stress, dry weight of the aerial part was measured every 3 days after imposing the stress. At the 6^{th} day of stress that dry weight declined more than 50%, samples were collected. The samples for proteomic analysis, collected from 35-day-old plants (with three replications for each group), were immediately frozen by liquid nitrogen, and stored at −20°C. All healthy leaves of one plant were considered as one sample, and a total of six samples (three for control and three for treatment group) were used to extract total protein. To measure dry weight, roots were removed and the aerial parts dried in an oven at 72°C for 24 h.

2.2 Total Leaf Protein Extraction

To extract total protein, 0.1 g of homogenized leaf sample was suspended in 1800 µl of Solution I (100 g of TCA, 700 mg of DTT, 1000 µl of deionized water) at −20°C for one hour and shaken every 15 min. After one hour, the sample was centrifuged (−4°C, 15000 RPM, and 20 min) and 1800 µl of Solution II (70 mg of DTT in 100 µl of deionized water) was added, and this process repeated for Solution I. The resulting pellet was washed three times and dried under hood, and then suspended in 250 µl of lysis buffer [5.1 g of urea, 1.9 Tiourea, 0.5 g of CHAPS, 0.1 g of DTT, 100 µl of Ampholin (3–10), and 0.06 g of Tris Base]. The suspension was shaken for 10 min under 40°C, and finally centrifuged at 15000 RPM at 24°C for 20 min. The protein concentration was measured according to Bradford [23].

2.3 2D Gel Electrophoresis of the Proteins

In order to profile protein patterns of drought-affected plants, three replicates from control and drought-treated plants were used. In the first dimension, the total protein extract (350 µl) was dissolved in IEF [1260 mg of Urea, 745 µl of H_2O, 525 µl of NP-40, 304.5 µl of Acrylamide stock 30%, 65.5 µl of Ampholin (5–8), 65.5 µl of Ampholin (3–10), 3.18 µl of APS 10%, and 2.62 µl of TEMED]. After 17.5 h of electrophoresis at 200 (30 min), 400 (16 h), and 600 (1 h) volts, the resulting gel was equilibrated twice for 15 min at 10 ml of equilibration buffer (10 ml of Glycerol 85%, 0.75 g of Tris, 2.5 g of SDS, and 5 ml of β-mercaptoethanol). In the second dimension, proteins were resolved in 30%

SDS-polyacrylamide gels (SDS-PAGE) and the wattage was 30 mA. Three biological replicates were conducted for each group (three for control and three for stressed group).

2.4 Gel Image and Statistical Analysis

The gels were incubated on a shaker in 5 g of Coomassie Brilliant Blue (CBB), 1250 ml of methanol, 250 ml of acetic acid, and 2.5l of water for 1.5 h, and then washed overnight in a solution of 916 ml of methanol and 250 ml of acetic acid. A Bio-Rad GS-800 densitometer was used to scan the gels. Melanie 2D gel analysis software was used to quantify the spots. The protein spots between the control and stressed gels were matched automatically; however, manual edition was performed to correct mismatches, especially in the arrears with high-clustered spots. Protein spots relative volume (%vol) was quantified and used to identify significantly changed spots. This parameter is relatively independent of variation due to protein loading and staining. Statistical significance of the relative change of accumulation of protein spots was determined using Student's *t*-test. Those protein spots that were recognized as differentially abundant proteins (DAPs) compared to the control groups were identified as described in [20].

2.5 Construction of Protein-Protein Interaction Network

STRING 11.0 (https://string-db.org/) is an open source online bioinformatics tool that was used for constructing protein-protein interaction network (PPI). The data setting was set as follows; minimum required interaction score: highest confidence (0.900), organism: *Arabidopsis thaliana* L. Constructed PPI was downloaded and visualized using Cytoscape 3.8.2 [24]. Due to a lack of annotation data for the majority of *Brassica napus* proteins in the STRING database, this study used ortholog of identified DAPs from *Arabidopsis*. The PPI of identified DAPs was generated consisting of all DAPs and their interactions. To gain more insight at the system level, we also generated the extended network (up to the 4^{th} extended network) for DAPs (confidence level: score ≥ 0.70) to identify their neighbors. KEGG pathway analysis for neighbors was performed to detect significant biological pathways (FDR > 0.05) associated with DAPs, based on false discover rate.

3 Results

3.1 Proteome Profile of Drought-Stressed Plants

To investigate proteome changes of drought stressed-plants, comparative analysis was carried to identify proteins that their expression significantly ($P < 0.05$) altered in response to drought stress. To ensure that plants experienced severe stress, the dry weight of the aerial part was measured and the result showed the 57.6% decrease in 35-day-old plants ($P < 0.05$). Analysis of gels from control and stressed plants showed 161 reproducible spots. As shown in Fig. 1, using t-student test, analyzing %vol of protein spots indicated 21 differentially abundant spots ($P < 0.05$). The spot 2 and 14 showed increased abundance in plants exposed to drought stress while the abundance level of reaming spots showed a significant decrease (Table 1). All differentially abundant spots were identified and the results were detailed in Table 1.

3.2 Protein-Protein Interaction Network

At first step, we constructed PPI of DAPs and considered these proteins as seed proteins in the network. This network consisted of 21 nodes (DAPs), 17 edges, and 2.66 average numbers of neighbors. There were 14 connected nods and 7 disconnected nodes. The connected nodes were in two groups, including 12 nodes with 16 edges and 2 nodes with 1 edge (Fig. 2).

At second step, to get more insight we extended the network of DAPs with decreased abundance 11 connected nods in the first group (red circles in Fig. 3), 2 connected nodes in the second group (red rectangles in Fig. 4), and 6 disconnected nodes in third group (red triangles in Fig. 5) and DAPs with increased abundance (the fourth group represented as green diamonds in Fig. 2).

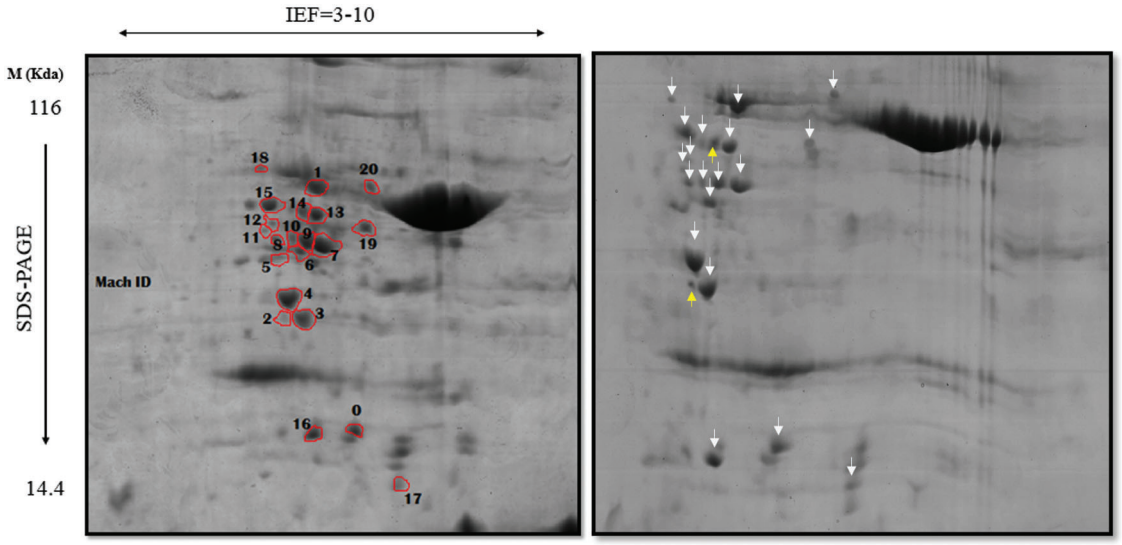

Figure 1: Differentially abundant proteins (DAPs) in the leaves of canola under control (left) and treated samples with severe drought stress (right). Spot numbers refer to IDs of identified proteins detailed in Table 1. Total protein of canola leaves (350 µl) was separated in two dimensions. First according to the isoelectric point and then according to the size of proteins. Up and down arrows show increased and decreased abundance

Table 1: A list of differentially abundant proteins identified in canola leaves under drought stress. IDs are according to Fig. 1

ID	pI	M (Kda)	Accession number	Description	Organism	Gene	Ortholog	Fold changes
0	6.16	25	A6NA23	Superoxide dismutase [Fe] 2, chloroplastic	*Brassica napus* (Rape)	FeSOD	AT5G23310	−0.54
1	5.65	58	A0A078FIS5	Probable bifunctional methylthioribulose-1-phosphate dehydratase/enolase-phosphatase E1	*Brassica napus* (Rape)	BnaA10g07100D	AT5G53850	−0.71
2	5.25	37	Q09052	1-aminocyclopropane-1-carboxylate oxidase	*Brassica* Spp.	ACO	AT1G62380	+2.11
3	5.5	37	A0A078H163	Pyruvate dehydrogenase E1 component subunit alpha	*Brassica napus* (Rape)	BnaA09g51510D	AT1G01090	−0.55
4	5.3	40	A0A078EZL5	Pectatelyase	*Brassica napus* (Rape)	BnaC06g15490D	AT3G54920	−0.66
5	5.18	46	M1F2H2	Chloroplast ribulose-1,5-bisphosphate carboxylase/ oxygenaseactivase	*Brassica* Spp.	RCA	AT2G39730	−0.60

(Continued)

Table 1 (continued)

ID	pI	M (Kda)	Accession number	Description	Organism	Gene	Ortholog	Fold changes
6	5.46	46	P92979	5'-adenylylsulfate reductase 1, chloroplastic…	*Arabidopsis thaliana*	APR1	AT4G04610	−0.64
7	5.74	47	Q8RUW5	Serine carboxypeptidase-like 8	*Arabidopsis thaliana*	SCPL8	AT2G22990	−0.75
8	5.15	49	Q9C9C4	Enolase 1, chloroplastic.	*Arabidopsis thaliana*	ENO1	AT1G74030	−0.72
9	5.54	48	A0A078FBY5	Vacuolar cation/proton exchanger	*Brassica napus* (Rape)	BnaCnng03980D	AT5G01490	−0.63
10	5.35	49	A0A078H3P0	Ureidoglycolate hydrolase	*Brassica napus* (Rape)	BnaC04g44790D	AT2G35810	−0.65
11	5	50	A0A078I4Y4	Tubulin alpha chain	*Brassica napus* (Rape)	BnaA02g04460D	AT4G14960	−0.57
12	5.11	51	Q0WMR0	Jacalin-related lectin 12	*Arabidopsis thaliana*	JAL12	AT1G52120	−0.69
13	5.67	53	A0A078FUB8	Adenosylhomocysteinase	*Brassica napus* (Rape)	BnaA01g23510D	AT3G23810	−0.63
14	5.49	54	O23240	D-2-hydroxyglutarate dehydrogenase, mitochondrial	*Arabidopsis thaliana*	D2HGDH	AT4G36400	+1.77
15	5.07	55	Q9MS49	ATP synthase subunit beta, chloroplastic	*Brassica napus* (Rape)	atpB	ATCG00480	−0.65
16	5.63	25	Q2EGT6	Glutathione S-transferase	*Brassica napus* (Rape)	GST	AT1G78370	−0.68
17	6.75	20	Q9FG59	Probable glutathione S-transferase DHAR4	*Arabidopsis thaliana*	DHAR4	AT1G75270	−0.57
18	4.95	62	Q9SKC3	Probable E3 ubiquitin-protein ligase ARI9	*Arabidopsis thaliana*	ARI9	AT2G31770	−0.75
19	6.3	51	Q71SX0	Ribulosebisphosphate carboxylase large chain	*Brassica napus* (Rape)	rbcL	ATCG00490	−0.56
20	6.36	59	Q6YSM3	Cytochrome c oxidase subunit 1	*Brassica napus* (Rape)	cox1	ATMG01360	−0.63

Note: *Abbreviations: pI: Isoelectric point; M (Kda): Protein mass weight (Kilo Dalton).
**Accession number of proteins in UniProtKB.

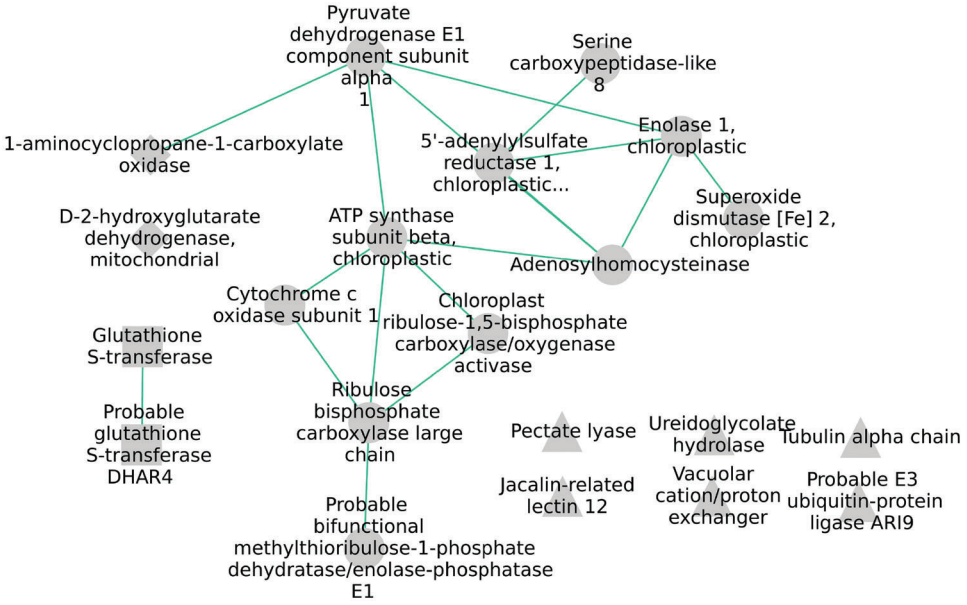

Figure 2: The PPI network of identified DAPs in canola leaves under severe drought stress. The network includes all DAPs and interactions between them. PPI and extended PPI of DAPs constructed using STRING 11.0 with confidence score of >0.70

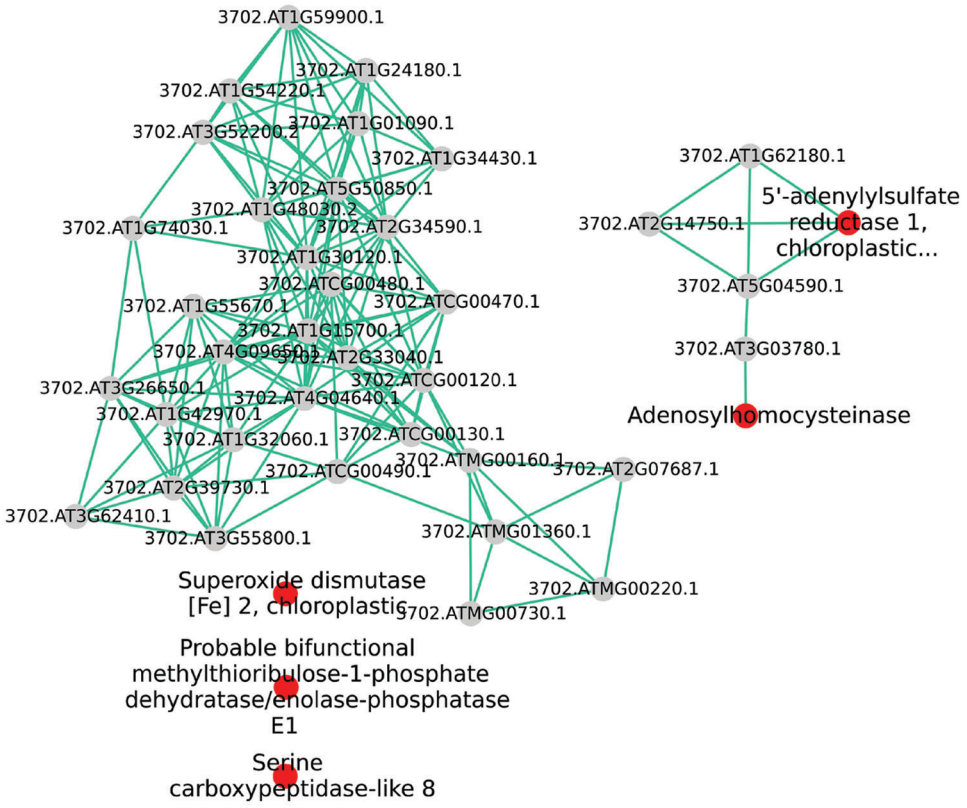

Figure 3: The 4th extended network of the first group of DAPs and their interactions with neighbors. PPI and extended PPI of DAPs constructed using STRING 11.0 with confidence score of >0.70

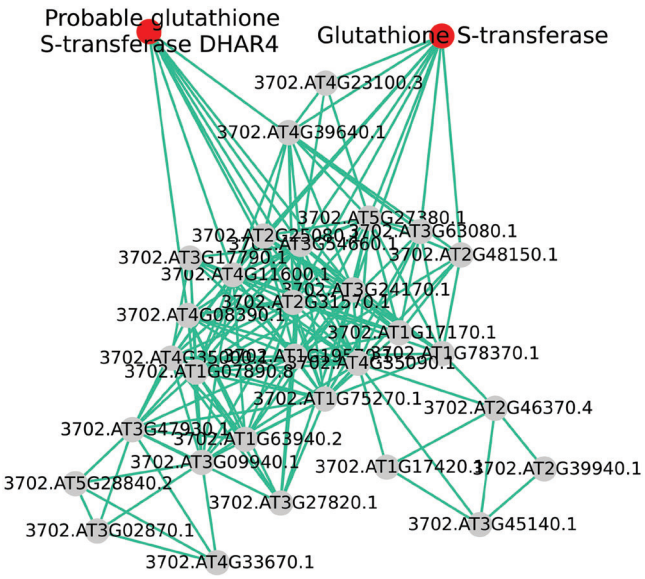

Figure 4: The 4th extended network of the second group of DAPs and their interactions with neighbors. PPI and extended PPI of DAPs constructed using STRING 11.0 with confidence score of >0.70

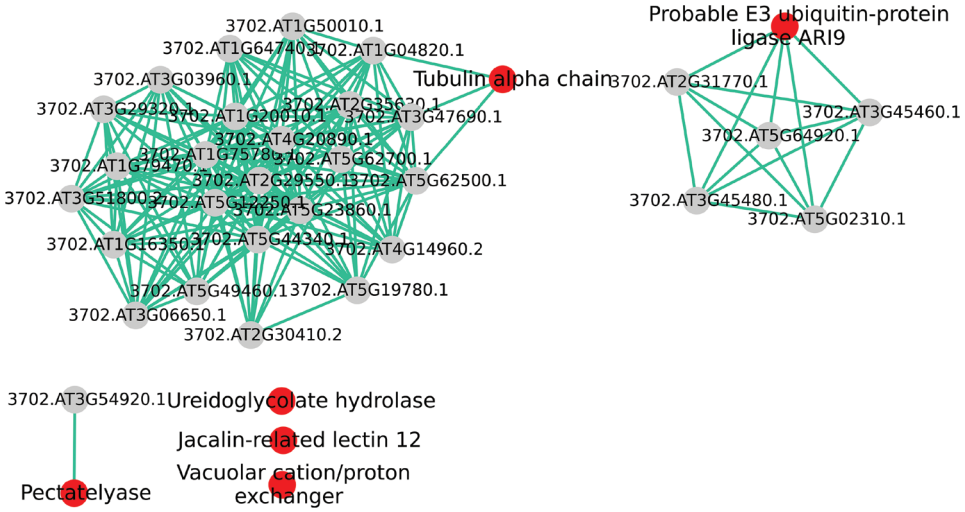

Figure 5: The 4th extended network of the third group of DAPs and their interactions with neighbors. PPI and extended PPI of DAPs constructed using STRING 11.0 with confidence score of >0.70

The network of the first group was extended to the 4th network. This network consisted of 41 nodes, 163 edges, and an average number of 9.68 neighbors. KEGG analysis of neighbor showed 12 significantly enriched pathways [Metabolic pathways, Carbon metabolism, Oxidative phosphorylation, Citrate cycle (TCA cycle), Glycolysis/Gluconeogenesis, Pyruvate metabolism, Photosynthesis, Biosynthesis of secondary metabolites, Carbon fixation in photosynthetic organisms, Sulfur metabolism, Cysteine and methionine metabolisms, and Glyoxylate and dicarboxylate metabolisms]. The second group was also extended to the 4th network, and the result showed that the network consisted of 32 nodes, 168 edges, and an average number of 10.50 neighbors. KEGG analysis of neighbors in this network resulted in 10 significant pathways (Glutathione metabolism, Ascorbate and aldarate

metabolisms, Arachidonic acid metabolism, Metabolic pathways, Linoleic acid metabolism, Taurine and hypotaurine metabolisms, Biosynthesis of secondary metabolites, alpha-Linolenic acid metabolism, Cyanoamino acid metabolism, and Cysteine and methionine metabolisms). The extended network of the third group of DAPs with decreased abundance (disconnected nodes in the PPI network of seed proteins) included 36 nodes, 206 edges, and an average number of 14.96 neighbors. The neighbor nodes of this network were enriched to 4 KEGG pathways [Phagosome, Citrate cycle (TCA cycle), Biosynthesis of secondary metabolites, and Purine metabolism].

The fourth group was two DAPs with increased abundance. At the 4th extended network, two sets of highly connected nodes were identified but no connection detected between these sets. Therefore, the extended network of this group continued until to the detected nodes that connected these two sets of proteins. At the 6th extended network, two sets of proteins connected to each other. This network consisted of 62 nodes, 388 edges, and an average number of 12.51 neighbors (Fig. 6). KEGG analysis showed 21 significant pathways (Cysteine and methionine metabolisms, Metabolic pathways, Valine, leucine and isoleucine degradation, Biosynthesis of secondary metabolites, MAPK signaling pathway-plant, Propanoate metabolism, Butanoate metabolism, Synthesis and degradation of ketone bodies, Fatty acid metabolism, Fatty acid degradation, Arginine and proline metabolisms, Plant hormone signal transduction, Pyruvate metabolism, Pantothenate and CoA biosynthesis, Lysine degradation, Carbon metabolism, Plant-pathogen interaction, Tryptophan metabolism, Terpenoid backbone biosynthesis, Glyoxylate and dicarboxylate metabolisms, and Peroxisome) (Fig. 7).

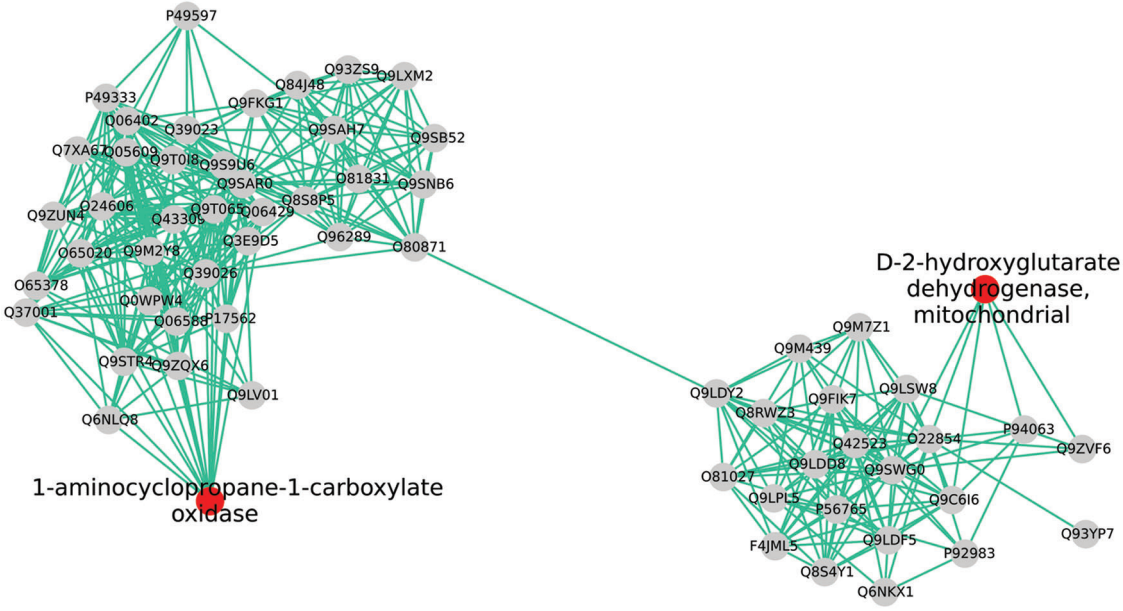

Figure 6: The 6th extended PPI network of 1-aminocyclopropane-1-carboxylate oxidase (Q09052) and D-2-hydroxyglutarate dehydrogenase, mitochondrial (O23240). This network includes two DAPs with increased abundance under drought stress and their neighbors from STRING database. The extended network was constructed using STRING 11.0 with a confidence score of >0.70

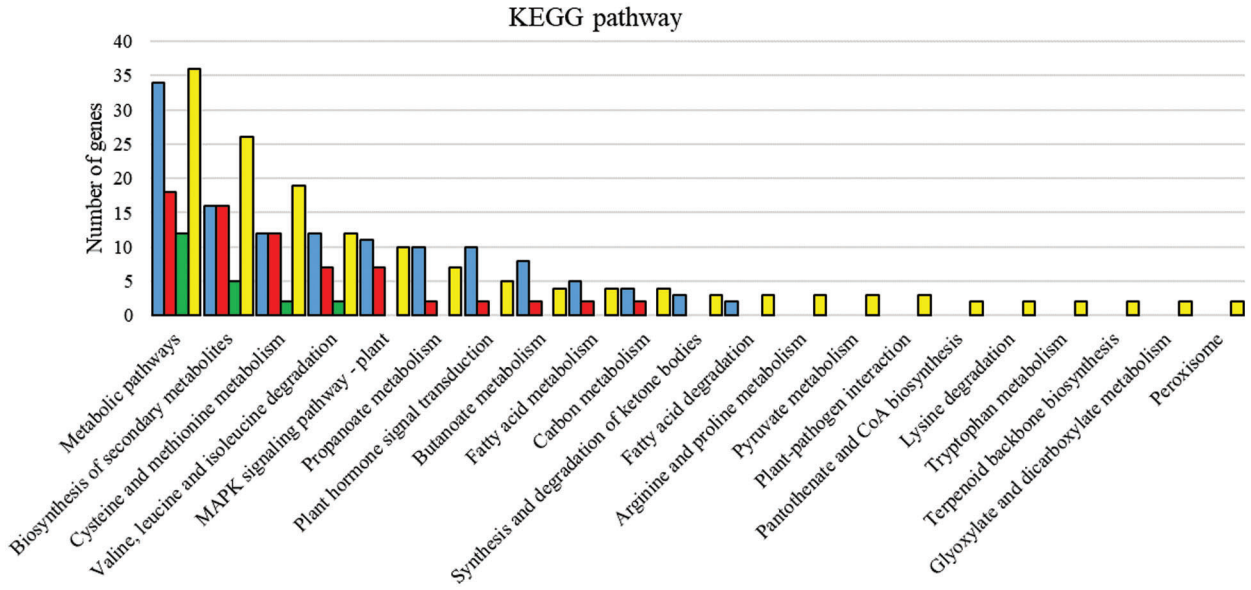

Figure 7: KEGG analysis of the extended networks. The blue, red, green, and yellow bars indicate the first, second, third, and fourth groups, respectively

4 Discussion

There are a little number of proteomics studies about canola-drought interactions and only a few have previously explored the proteomics profile of canola leaves under drought stress, mainly focused on tolerant cultivars and a little attention given on susceptible cultivars [25–27]. Previous findings have revealed that DAPs have a dynamic behavior and their abundance patterns depend on stress severity and duration, types of tissue, and growth stages [25–28]. The proteomic results of the present study support those of the previous findings. Here, we used a proteomic profiling approach and constructed PPI network of DAPs and their neighbor nodes to widen our understanding of canola-drought interactions. We think that susceptible cultivars employ mechanisms that make them vulnerable to drought stress. To the best of our knowledge, this study explores molecular mechanisms responsible for the susceptibility to drought stress in canola (*B. napus* L.). The present study used DAPs as the backbone nodes to construct PPI up to 6th level for further comprehensive analysis. Most two-dimensional electrophoresis methods could resolve a limited number of protein spots, and may be most of promising proteins could not be studied. PPI network and extend PPIs could reveal much more proteins and biological processes.

Interestingly, two proteins showed increased abundance under drought stress (Table 1 and Fig. 1). The enzyme 1-aminocyclopropane-1-carboxylate oxidase (Q09052 or ACO) is the second ethylene biosynthesis protein, which converts ACC into ethylene. According to previous studies, ACO is a rate-limiting enzyme of ethylene biosynthesis [29–31]. A significant higher level of this enzyme could imply the over production of ethylene in canola leaves. In accordance to our results, several studies have shown the significant increase in the abundance level of ACO under biotic/abiotic stresses [32–34]. It has already been shown that ethylene modulates oxidative stress in plants, and in plant mutants to ethylene the activity of antioxidant enzymes is comparatively higher than that on wild-type plants [35]. (R)-2-hydroxyglutarate dehydrogenase (O23240 or D2HGDH) catalyzes oxidation of d-2-hydroxyglutarate (d-2HG) to 2-oxoglutarate which can enter the TCA cycle and transfers its electron to the electron transport chain through the ETF-ETFQO complex [36]. Previously, it has been indicated that this protein is increased during developmental and dark-induced senescence in *Arabidopsis thaliana* [37]. Engqvist et al. [36] showed that more demand for carbon during development and dark-induced senescence leads to use alternative substrates such as carbon derived from

degradation of amino acids, lipids, etc. D2HGDH participates in β-oxidation and degradation of amino acids alongside degradation of chlorophyll, and acts as a donor of electrons [36]. It has well been indicated that under drought conditions many processes are interrupted, especially photosynthesis, causing the cell use their own components such as proteins, amino acids, and lipids as carbon sources [38,39]. We think that an increase of D2HGDH expression is to use alternative substrates as carbon sources to compensate the carbon shortage under drought stress.

PPI network of ACO and D2HGDH showed that these proteins are not directly connected (Fig. 6). The 6^{th} extended network showed that the sub network of ACO and D2HGDH is connected through probable protein phosphatase 2C 25 (O80871) and 2-oxoisovalerate dehydrogenase subunit beta 2 (Q9LDY2). These proteins are involved in abscisic acid signaling, response to nutrition starvation, and response to drought stress [40,41]. Moreover, we identified 21 pathways associated with ACO and D2HGDH, of which, 15 pathways were unique. The majority of ACO and D2HGDH neighbors (45.36%) were enriched in three pathways including valine, leucine and isoleucine degradation, MAPK signaling pathway, and propanoate metabolism. Valine, leucine and isoleucine degradation is one of well characterized plant responses to osmotic stress in leaves [42]. MAPK signaling pathway- as a positive regulator under drought stress- and proteins related to the propanoate metabolism have previously been indicated in response to drought stress in other species [43,44].

This study showed that the abundance of 19 proteins was decreased in response to drought stress in the canola susceptible cultivar (Fig. 1 and Table 1). Most of these proteins showed an increased abundance in previous reports, and some showed both increased or decreased levels in canola and other species under drought or other types of abiotic stresses.

Decreased abundance of chloroplastic superoxide dismutase (A6NA23 or SOD) and glutathione-S-transferase (Q2EGT6 and Q9FG59 or GST) is an interesting observation. One of the common events under abiotic stresses is the production of reactive oxygen species (ROS). In response to ROS production, plants commonly use antioxidant enzymes, including SOD and GST [45]. Our results showed the decreased levels of SOD and GST under drought stress. However; many studies have indicated that the high level of SOD under abiotic stresses increases the plant tolerance to stresses [46–49]. The significant lower level of SOD and GST under drought stress could be one of the reasons of susceptibility to drought stress. This is because under the condition that ROSs are in a high level and antioxidant enzymes are in a low level, ROSs will damage the cell essential components.

The results showed the decreased abundance of 5 DAPs related to photosynthesis and energy production. It was reported in canola tolerant cultivars that ATP synthase subunit beta (Q9MS49) and Ribulosebisphosphate carboxylase large chain (Q71SX0) are increased under drought stress [25,26]. Moreover, previous findings have found that high levels of Pyruvate dehydrogenase E1 component subunit alpha (A0A078H163), Chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenaseactivase (M1F2H2), and Cytochrome c oxidase subunit 1 (Q6YSM3) are associated with tolerance to abiotic stresses [50–52]. Among these proteins related to photosynthesis and energy production, Pyruvate dehydrogenase (A0A078H163 or PDC) has a central role. The PDC is a multienzyme consisting of three primary components, including pyruvate dehydrogenase (E1), dihydrolipoyl acetyltransferase (E2) and dihydrolipoyl dehydrogenase (E3) [53]. PDC is a main regulatory enzyme and catalyzes oxidative decarboxylation of pyruvate into acetyl-CoA and NADH. Following that, acetyl-CoA enters to the TCA cycle to produce energy or be used for synthesizing fatty acids [54].

KEGG analysis of the extended networks of DAPs showed that these proteins are connected with 21 pathways. All of these pathways have previously been characterized for being involved in response to biotic and abiotic stresses in crops [55–57].

5 Conclusion

Identification of molecular components and understanding the in-depth mechanisms under altered conditions could give valuable information in developing drought tolerance. This study found that DAPs with increased abundance were associated with degradation processes, while DAPs with decreased abundance were mainly connected with energy production, ROS scavenging, and macromolecules production such as amino acids and nucleotides. Our investigation will help to elucidate the molecular basis of canola susceptibility to drought stress.

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