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Determination of Metabolic pathways and PPI network of Sarigol in Response to Osmotic stress: An in silico study

Reza Shokri-Gharelo[1*], Ali Bandehagh[1], Bizhan Mahmoudi[2]
[1]Department of Plant Breeding and Biotechnology, University of Tabriz, Tabriz 51666-16471, Iran
[2] Department of Animal science, Ramin Agriculture and Natural Resources University, Ahvaz, Iran

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ABSTRACT

The complexity of plants response to abiotic stress make difficult to manage and target special genes/proteins to be used in improving crop performance. Therefore, understanding and insight into molecular mechanisms recruited by plants under stressful conditions is essence. In this aim, Sarigol, a salt-sensitive cultivar of canola, based on their differentially expressed proteins was studied in silico. The results indicated that the majority of proteins had molecular function of catalytic activity and involvement of these proteins in response to stress underrepresented by Sarigol, whereas proteins involved in cellular and metabolic process were overrepresented. Phylogenetic analysis divided the proteins into 4 groups and protein-protein interaction network prediction illustrated two sets of interacted proteins, while most of proteins did not show any interactions. The results suggested that in the molecular level, Sarigol is unable to respond appropriate actions as are observed in tolerant plants.

Corresponding Author:

Reza Shokri-Gharelo,
Department of Plant Breeding and Biotechnology, University of Tabriz, Tabriz 51666-16471, Iran

Email: gharelo.reza@yahoo.com

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1. INTRODUCTION

The plant cells respond to variety of abiotic stress with complex mechanisms in which wide range of genes and proteins are involved. The eventual effect of stress is reduction in growth and productivity of plants in morphological level and changing gene/protein expressions at molecular level [1-3]. The molecular, biochemical, and physiological responses of plants are different upon encountering various stress conditions [4-6]. Plants have to customize their response at molecular and cellular level to be able to adapt with stressful conditions. In the molecular level, plants trigger significantly two series of proteins in response to abiotic stress. First, functional proteins that include water channels, transporters, protection factors of macromolecules (LEA proteins, chaperons), proteases and second, regulatory proteins consist of transcription factors, protein kinases, phosphatases, phospholipid metabolism, and ABA biosynthesis [7, 8]. There are many reports that indicate introducing the gene of these functional or regulatory protein into plants confers them more stress tolerance, leading to creating of improved crops with the better tolerance/resistance under stress conditions [9, 10].

The stress-tolerant plants are a result of appropriate recruiting of biological pathways and up/down regulation of specific genes/proteins. Understanding these mechanisms and identifying genes/proteins involved in interactions of plant-stress are critical step to reach tolerant plants, especially tolerant crops for securing food production [11, 12]. Recently, the development of novel computational tools and algorithms has paved the way of studying structure, function, and interaction network of proteins and metabolic pathways in which stress-responsive proteins present. This study investigates osmotic stress-responsive proteins in one of the Canola susceptible cultivars, Sarigol. These proteins with differentially expression changes were selected from our previous study [13]. Determination of biological pathways, their interaction networks, and physicochemical

properties of the proteins in Sarigol would be helpful in our understanding and further insight of its molecular mechanisms under sever osmotic stress conditions.

2. RESEARCH METHOD

2.1. Input data

We used proteins that identified as differentially expressed proteins by two-dimensional gel electrophoresis technique under extreme osmotic stress, in the aim of studying the biological pathways, molecular functions, and protein-protein interaction networks that are affected by the stressful conditions. Proteins are final products of genes and could be used as the best indicator of biological pathways that are mostly activated and overrepresented by an organism in the different conditions of environment. All of proteins were blasted against TAIR (The Arabidopsis thaliana Information Resource) protein database, due to all of these protein identifications were performed based on different organisms. Obtained homologous proteins were used as entries to predict biological pathways, molecular functions, protein-protein interaction networks, and their structural characteristics.

2.2. Biological pathway prediction

BiNGO (The Biological Networks Gene Ontology tool) in Cytoscape, open source software platform, were used to study and visualize biological pathways and molecular functions of studying proteins [14]. BiNGO is a java-based software to determine the significance of gene ontology (GO) that are overrepresented among the set of genes [15]. The setting of BiNGO was set with following parameters: Hypergeometric test selected for statistical test, 0.01 selected for a significance level, and Arabidopsis thaliana selected for organism/annotation.

2.3. Protein-protein interaction network prediction

STRIN 10.0 (http://string-db.org/) is an open source online bioinformatics tool that used for predicting and studying protein-protein interaction network (PPI). The data setting was set as follows; minimum required interaction score: highest confidence (0.900), organism: Arabidopsis thaliana and disconnected node were hidden from the network. The most interacted proteins were determined for Sarigol. In addition, the gene counts in KEGG pathways determined to specify affected pathways.

2.4. Phylogenetic analysis

Phylogeny.fr-web based and free online tool was used to construct and analysis phylogenetic tree for osmotic stress-responsive proteins in Sarigol [16]. First, all of proteins aligned by CLUSTALW and then phylogenetic tree constructed. This tool constructs phylogenetic tree by PhyML and visualizes by TreeDyn.

3. RESULTS AND ANALYSIS

3.1. Overrepresented molecular function, cellular components, and biological processes in Sarigol

The p-value of 0.05 was considered to determine significantly overrepresented molecular function, biological process, and cellular components (Table 1 and Figure 1). Molecular function analysis indicated that 68.7% of these proteins are significantly participated in catalytic activities (Table 1 and Figure 1). The majority of proteins present at cytoplasm of cell (62.5%) and plastid (43.7%) and only 18% present at extracellular regions.

Our studying proteins significantly (p-value of 0.05) were overrepresented in the five biological processes (Table 1 and Figure 1). Among these five biological processes, the mostly overrepresented processes were cellular process and metabolic process (Figure 2). The proteins involve in response to abiotic stress consist about 18% cluster frequency and about 4% of total frequency (Figure 2).

3.2. KEGG pathways and protein-protein interaction network of osmotic stress-responsive proteins in Sarigol

In the aiming to specify which of cellular pathways are more affected under osmotic stress in Sarigol, KEGG pathways were investigated and results revealed that osmotic stress-responsive proteins are involvement in cysteine and methionine metabolism (pathway ID: 00270) and carbon metabolism pathway (pathway ID: 00270). The count in gene set was observed 3 for each of these identified pathways.

As represented in Figure 3, the protein-protein interaction network construction by STRING 10 illustrated that two distinctive cluster of proteins show interactions. Four proteins include ribulose bisphosphate carboxylase small chain 1A, rubisco activase, ATP synthase delta-subunit gene, Fe superoxide dismutase 1 and three proteins including adenosylhomocysteinase 1, enolase 1, pyruvate dehydrogenase E1 beta consisted cluster 1 and 2, respectively. The link between cluster 1 and 2 did not observed (Figure 3).

Table 1. Molecular function, biological process, and cellular components of differentially expressed proteins in the leaves of Sarigol under osmotic stress.

GO-ID	Description	Corrected p-value	Cluster frequency	Total frequency
9628	Response to abiotic stress	1.61E-03	37.50%	4.20%
5737	Cytoplasm	1.66E-03	62.50%	17.10%
9536	Plastid	1.73E-03	43.70%	7.70%
6950	Response to stress	5.16E-03	37.50%	6.70%
3824	Catalytic activity	6.69E-03	68.70%	27.30%
9579	Thylakoid	6.69E-03	18.70%	1.10%
5576	Extracellular region	1.01E-02	18.70%	1.40%
5623	Cell	1.15E-02	81.20%	42.40%
5622	Intracellular	1.33E-02	62.50%	26.10%
9987	Cellular process	1.47E-02	62.50%	26.70%
6091	Generation of precursor metabolites and energy	2.69E-02	12.50%	0.70%
8152	Metabolic process	2.96E-02	56.20%	24.70%

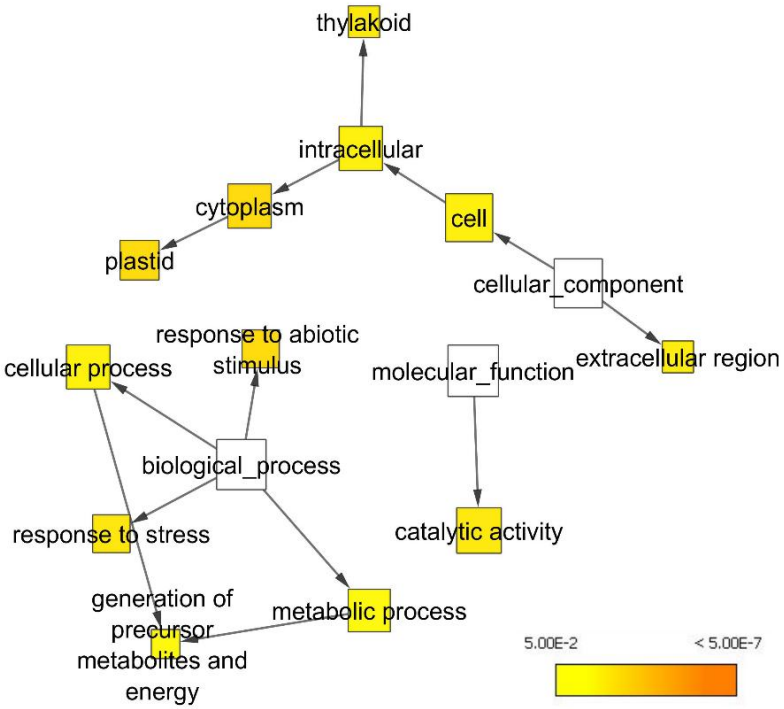

Figure 1. Schematic representation of Molecular function, biological process, and cellular components of differentially expressed proteins in the leaves of Sarigol under osmotic stress. The p-value of 0.05 considered as a statistically threshold in Bingo, a plugin in Cytoscape.

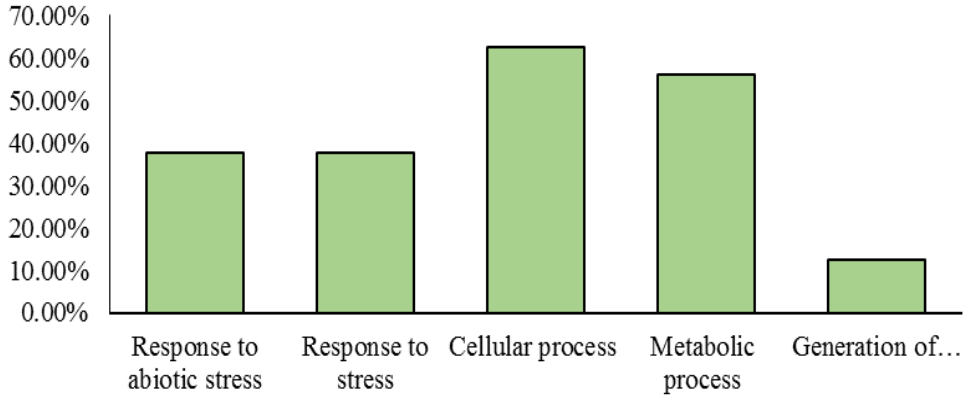

Figure 2. The cluster frequency of biological process of osmotic stress-responsive proteins in Sarigol leaf. The p-value of 0.05 was considered as the statistical threshold.

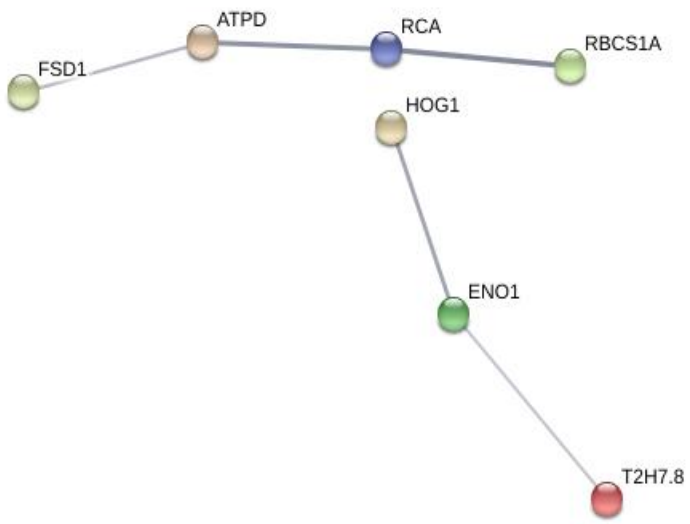

Figure 3. Protein-protein interaction network of osmotic stress-responsive proteins in Sarigol. The non-interacted proteins were hidden from results. Abbreviations: RBCS1A: ribulose bisphosphate carboxylase small chain 1A, RCA: rubisco activase, ATPD: ATP synthase delta-subunit gene, FSD1: Fe superoxide dismutase 1, HOG1: adenosylhomocysteinase 1, ENO1: enolase 1, T2H7.8: pyruvate dehydrogenase E1 beta.

3.3. Phylogenetic analysis of osmotic stress-responsive proteins in Sarigol

Phylogenetic tree for osmotic responsive-proteins was constructed using Phylogeny.fr. All of proteins divided into 4 groups in un-rooted tree (Figure 4). Group 1 includes only one protein, Q8VZU3. The number of nine proteins including Q9C9C4, P21276, Q9SKC3, Q8L7C9, Q8VXY9, Q9SSS9, P92979, Q39219, and Q9C5M8 located within group 2 and the number of seven proteins including Q9C6Z3, O23255, Q41931, Q9FN41, O23240, P10896, and P11139 located in group 3. Three proteins located into group 4, O04312, Q39253, P10795.

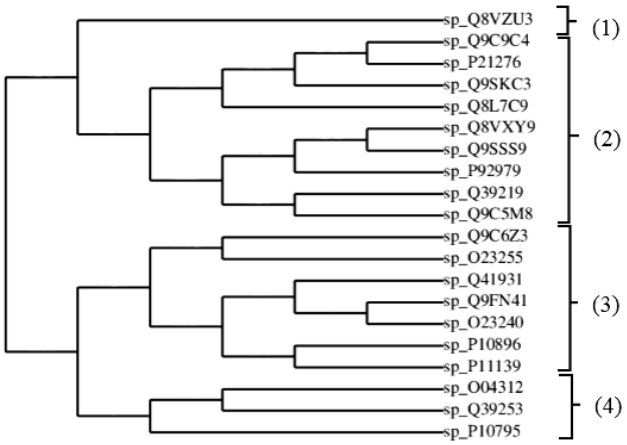

Figure 4. Phylogenetic analysis of osmotic-responsive proteins in Sarigol. Abbreviations: P21276: Superoxide dismutase [Fe] 2, Q9FN41: Probable bifunctional methylthioribulose- 1-phosphate dehydrogenase, Q41931: 1-aminocyclopropane-1-carboxylate oxidase 4, Q9C6Z3: Pyruvate dehydrogenase E1 component subunit beta, Q9C5M8: Pectate lyase 3, P10896: Ribulose bisphosphate carboxylase/oxygenase activase 1, P92979: 5'-adenylylsulfate reductase 1, Q8VZU3: Serine carboxypeptidase-like 8, Q9C9C4: Enolase 1, Q39253: Vacuolar cation/proton exchanger 1c, Q8VXY9: Ureidoglycolate hydrolase, P11139: Tubulin alpha-1 chain, O04312: Jacalin-related lectin 12, O23255: Adenosylhomocysteinase 1, O23240: D-2-hydroxyglutarate dehydrogenase, Q9SSS9: ATP synthase subunit beta, Q8L7C9: Glutathione S-transferase U20, Q9SSS9: ATP synthase subunit b, Q39219: Cytochrome c oxidase subunit 1, Q9SKC3: Probable E3 ubiquitin-protein ligase ARI9, P10795: Ribulose bisphosphate carboxylase large chain.

Sarigol is a salt-sensitive species of canola that affected severely upon encountering with stressful conditions [14, 15]. The mechanisms by which plants cope with stress conditions are complex [16] and essential to be known in order to manage methods for improving plant performances under unfavorable growth conditions. As mentioned above, the majority of our studying proteins have catalytic activity and take part in cellular and metabolic process and the minor of them participate in stress response (Table 1). In contrast, many studies on responsive genes/proteins in tolerant plants have been revealed that those genes/proteins with stress response activities and involved in energy metabolisms constitute the major portion of totally induced genes/proteins [17-22]. These results suggest that Sarigol could not able to orchestrate functionally appropriate proteins.

Metabolic pathways adjustment is elaborately regulated in response to stimuli and varied by species to species and type of stimuli. Profiling metabolic pathways could significantly help to understand stress-responsive mechanisms. The results indicated that osmotic stress-responsive proteins in the leaf of Sarigol participate in cysteine/methionine metabolism, and carbon metabolism. Between these pathways, the roles of Cysteine (Cys)/methionine (Met) metabolism have been taken weak attentions under sever conditions. Cysteine and methionine are two major amino acids that not only act as block for synthesizing proteins but also play many important roles in the cell [23]. One of the main importance of these amino acids is because of their sulfur moiety, which make these sulfur-containing amino acids a crucial player within the cell in time of abiotic stress [24]. Cysteine and methionine as a portion of antioxidant system could undergo ROS-mediated oxidation to scavenger reactive oxygen species [25-28]. Production of reactive oxygen species under stressful conditions is commonly observed event that could severely damage the cell essential components in the absence of suitable defense mechanisms, consequently, resulted in the cell death [29, 30]. Taking together the results of our previous study that indicated down regulation of osmotic stress-responsive proteins and KEGG pathways analysis, it is revealed that Sarigol have not ability to encounter with reactive oxygen species-caused damages. This expose the stress affected cells sustainable to be damaged and eventually disrupted, which are manifested on morphologic level as decreased growth characteristics.

In the conditions of osmotic stress based on differentially expressed proteins, two sets of interacted proteins observed in Sarigol. The number of 4 proteins in cluster 1 related to photosynthesis and ATP production as well as of 3 proteins in cluster 2 related to glycolysis. Meanwhile, no protein that links these

clusters together was observed (Figure 3). According to phylogenetic analysis in cluster 1, the ATPD and FSD1 located at group 2, RCA at group 3 and RBCS1A at group 4. In cluster 2, HOG1 plus T2H7.8 located at group 3 and ENO1 at group 2 (Figure 4). However, inferring mostly interacted proteins (hub) in our predicted interaction network is difficult because the number of interacted proteins is low. For introducing hub proteins, it is essential to identify more expression-affected proteins. These results may suggest photosynthesis and ATP production as mostly affected processes in Sarigol under osmotic stress. These processes efficiency are crucial to sustain growth and performance of plants under stressful conditions [31, 32].

4. CONCLUSION

In silico study of proteins with significantly changed level of expression were performed using bioinformatics tools to determine some molecular mechanisms recruited by Sarigol under osmotic stress. In summary, proteins with catalytic activity were overrepresented, whereas proteins involved in response to stress underrepresented. Mostly affected proteins belonged to Cys/Met and carbon metabolism. PPI network revealed two sets of interacted proteins only and most of proteins did not indicate any interactions and all of osmotic-responsive proteins divided phylogenetically into four groups.

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