

## **Study of protein profile of wheat bread in seedling stage using two-dimensional electrophoresis**

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### **Abstract**

Wheat is the first and foremost cereal crop in the world. The interval between the germination and early tillering stage is called seedling. The seedling stage, seed embryo has three initial leaf cells, which after germination, vegetative shoot apical meristem begins to be divided leading to the emergence of the leaves. In this study, proteomics has been used to study changes of protein profiles in wheat leaves in the different stages of seedling before tillering. To investigate changes in protein profiles, samplings took place in three stages: unifoliate, bifoliate and trifoliate. To study and identify the proteins associated with the seedling, total protein was extracted from wheat leaf in seedling stages by TCA-ACETON method and separated based on two-dimensional electrophoresis method. After analysis of gels obtained from the two-dimensional electrophoresis through Software Image master 6.0, 360 differentially expressed protein spots (DEPs) were detected. Among which, 31 protein spots showed significant changes in the level of 5%, in various stages of seedling. 50% of the identified proteins showed decreased expression and 19% showed increased expression and 31% of the identified proteins did not indicate significant regulatory changes.

**Keywords:** Proteomics, Seedling, Two-dimensional electrophoresis, Wheat

## Introduction

Cereals have a special and important role in the consumption patterns of each country and are an important food for humans. Almost 80% of proteins, 10% of fats, 40% of Glucosides and generally 80-90% of calories consumed by humans in the world are provided by cereals. In the meanwhile, wheat as an important commercial commodity in world and as a superior weapon in political and global relations has an increasingly strategic significance. Bread wheat as the humans' main food is of particular importance (Farahani, 1385). Wheat is a monocot plant from Graminae species and related to genus *Triticum* and has several important species among which *T.aestivum* can be pointed out. Bread wheat is Hexaploid ( $2n=6x=42$ ) and can grow in a wide range of weather conditions. It is in fact the world's most adaptable species of cereal crop and many fields are dedicated to it compared with other crop plants (Noor Mohammadi *et al.*, 1396).

A young leaf needs to increase complex light-collecting proteins in photosystems I and II and biosynthetic route enzymes to fulfill its photosynthetic capacity etc. (Feller and Fischer, 1994). Amino acids are the major carbon metabolites and nitrogen (Cabello *et al.*, 2006). Biosynthesis of amino acids is in the form of growth regulator and is active in the young parts of the plant. The content of amino acids varies depending on the physiological, genetic and environmental factors such as different intensity of the light (Ruuhola *et al.*, 2003). Plant growth is related to the processes related to production, transfer, and the use of the photosynthetic products. Young leaves grow fast, uptake nitrogen and carbon from other parts of the plant and intensify protein synthesis. In fact, with the maturity of the photosynthetic system, the leaf becomes a reservoir of carbohydrates. (Dertinger *et al.*, 2003) Proteins are privileged in comparison with other macromolecules, because they have maintained their connection with the genes and benefit from all catalytic and constructive properties and other necessary supplies to carry out their own actions. Therefore, proteins can be the best description for the action of individual genes at the molecular level. On the other hand, considering the fact that the response to environmental factors is performed by proteins increases the importance of the role of proteins in the cell. (Rossignolet *et al.*, 2006) In recent years, much research was done to identify parts of the model plants genomes such as Arabidopsis, rice and corn, but genome-related information is not alone indicative of the gene function in a particular stage of plant life and chemical and biological events that occur at any stage. To investigate these processes and complement previous works, applying the methods that specify quality and quantity of the expressed product by a gene is necessary. Today, in the level of transcriptome, proteome and metabolome, we can perform such analyses. Although we can perform qualitative transcriptome analyses using Microarray and SAGE methods, but quantitative levels of mRNA and protein are not consistent as a linear relationship. However, protein patterns can directly be used to check the components involved in a biological process and their quantitative levels. In fact, the proteins with enzymatic, regulatory and structural roles are the final product of transcribing parts of the genome that can be assessed and recognized in this method. Progresses made in the two-dimensional electrophoresis, increase protein sequence database and the use of mass spectrometry to identify proteins have made proteomics method a powerful instrument to detect total protein expressed in a variety of conditions (Mehrabi *et al.*, 2013) Proteomics is a very accurate science in the identification of proteins, their use and how to express them. Moreover, it is one of the best ways to determine accurate and valuable information in the preparation of concurrent information from these processes. Therefore, it can be used in the analysis of processes related to the leaf growth. In this study, changes in the proteome profile of bread wheat were studied in different developmental stages and the protein changes can be examined in this step according to the importance of this stage of the plant growth in production.

## Materials and methods

This research was conducted on wheat (Variety. Azar 4). The specific conditions of climate and the number of climates have faced agriculture of dryland areas with many problems. Given the importance of wheat and its role in community nutrition, the Dry Land Agricultural Research Institute (DARI) of Kermanshah-IRAN reformed and introduced bread wheat (Var. Azar4). Azar 4 is the result of a cross between wheat varieties of Sefid and line S Bb / Inia / Kvz / my 44 / Maya. After hybridization, line Kvz/

my ٧٧ / Maya S under the title of Azar ٢ was introduced to be cultivated ١n ١٩٩٨ in the cold and temperate dryland zones of the country due to enjoying special agronomic characteristics. Azar ٢ cultivar, due to traits such as early maturity, tolerance to drought stress, coldness and having more performance was selected compared to the control varieties such as Sefid and Sabalan (١٤ and ١٦٪) respectively. Besides it was planted in greenhouse conditions in vase. Harvested samples were used in unifoliate, bifoliate and trifoliate stages as the experimental material for the research. The samples were frozen in liquid nitrogen and were transferred to the freezer at -٨٠ °C until extraction of protein.

Protein extraction

All laboratory Experiments were done in Medical Biology Research Center(MBRC) of Kermanshah-TRAN. Protein extraction was done using Damerval and Mostafaei methods with little modification. (Mostafaie, ٢٠٠٣; Damervalet *al.*, ١٩٨٩) To precipitate protein and rinse on each tube, about ١٠ times more than the volume of supernatant solution of cold protein, (TCA ١٠ g, ٢٠ mmoldithiothreitol and acetone up to ١٠٠ ml) was added, vortexed and held for an hour in the freezer at temperature of -٢٠ °C. The samples were taken out of the freezer and centrifuged at temperature of ٤ °C for ٢٠ minutes at a speed of ١٣,٥٠٠ rpm. Rinse stage was repeated for ٥ times. After discarding the last supernatant, the tubes were held at room temperature for ٢٠ minutes to dry deposition. To dissolve protein, per ٢٠ mg of sediment, ٢٠٠-٣٠٠ micro-liters of two-dimensional buffer Lysis (Table ١) and ٢-٣ micro liters of pmsf were added. Then the precipitate was dissolved well and placed on a shaker for ١ hour at room temperature.

Table ١. Two-dimensional buffer Lysis	
Chemicals	The amount of the chemicals
Urea	٨ M
Tiurea	٢ M
CHAPS	٤٪
Tris	٣٥ mM
DTT	٨٠ mM
IPG Buffer	Maximum ٢٪

Tubes were centrifuged at room temperature for ٢٠ minutes at a speed of ١٣,٥٠٠ rpm. A little amount of supernatant was separated for determining the concentration (about ٥٠ ml). To measure the concentration of proteins in the test sample, the Bradford’s method (Bradford, ١٩٧٩) was used and the sample absorbance was read at ٥٩٥ nm.

Two-dimensional electrophoresis

In the first dimension, in order to perform electrophoresis, the ١٣-cm gel strip (IPG Strip) with linear pH = ٣-١٠ produced by the company Bio Rad was used. Complete rehydration of gel strips was conducted during the night with rehydration solution. Then the first dimension was performed with IPG phor٣ and at ٢٠ ° C with a specified program (Table ٢). To load the gel, ٥٢ thousand volt hours (٥٢KVh) is required. This stage lasted for ١٠: ٣٠ hours with the current strength of ٧٥mA.

Table ٢- Focusing program used by multi-Feb		
Time	Voltage	Program
١٥ Mins	٥٠٠ Volt	Gradient
١ Hour	٥٠٠ Volt	Step
١ Hour	٣٠٠٠ Volt	Gradient
١:٣٠ Hour(s)	٣٠٠٠ Volt	Step
٢ Hours	٨٠٠٠ Volt	Gradient
٣:٤٥ Hours	٨٠٠٠ Volt	Step

After the first dimension, gel strips were equilibrated for ۳۰ minutes in the first equilibration solution (Table ۳) containing DTT ۱٪. The first solution is completely discarded and strips are washed with double distilled water, placed in the second solution containing % ۰ idoacetamide and put into the shaker for ۳۰ minutes.

Table ۳- equilibration solution

Chemicals	The amount of the chemicals
۷,۰Tris-HCLpH=	۳۰ mM
Urea	۷ M
Glycerol	۳۰٪
SDS	۲٪
Bromophenol Blue	Multi-crystal

Then the strip was placed on acrylamide gels (Mostafaie, ۲۰۰۳) at a concentration of ۱۱٪. For making acrylamide gel, the lower gel buffer was prepared according to the Table (۴) and Stoke acrylamide was prepared according to Table (۵).

Table ۴. Low gel buffer (pH<sup>۸</sup> / ۸)

Chemicals	The amount of the chemicals
Tris	۱۸,۲ grams
SDS	۰,۴ grams
Distilled water	Up to ۱۰۰ ml

Table ۵: Stoke acrylamide

Chemicals	The amount of the chemicals
Acrylamide	۳۰ grams
Base Acrylamide	۰,۸ grams
Distilled water	Up to ۱۰۰ ml

Then the materials were mixed together according to (Table ۶). During this stage, the gel containing ۰٪ agarose in buffer electrode (۳ alkaline Tris ۳ grams, Glycine ۱۴,۴ g and SDS ۱ g) was prepared and heated until dissolved. Then melted agarose was poured on IPG strips to create a well-established connection between strip and gel. Electrophoresis device is turned on with the considered program, running gel was continued until the color of Bromophenol **Blue** reached to ۱ to ۳ mm of the end of the gel.

Table (۶)-- Ingredients for preparation of ۳۰ ml of acrylamide gel ۱۱٪

Chemicals	The amount of the chemicals
Lower gel buffer	۷,۰ ml
Ionized distilled water	۱۱,۴۹ ml
Acrylamide	۱۰,۷۱ ml
TEMED	۱۰۰ micro liter
Ammonium persulfate	۱۰۰ micro liter

After completion of electrophoresis, the staining was done according to Blum protocol (Blum et al., ۱۹۸۷) About ۲۰۰ ml of Coomassie Brilliant Blue R-۲۰۰ was also added (Table ۷). This step is recommended for at least ۱۶ hours.

Table (۷)-Coomassie brilliant blue dye-solution R-۲۰۰

Chemicals	The amount of the chemicals
Stock Coomassie Brilliant Blue R-۲۰۰	۱۰۰ ml
Glacial acetic acid	۸۰ ml
Distilled water	۲۰ ml

Then ۲۰۰ ml bleaching solution was poured on gel containing ۴۰ ml of methanol, ۲۰ ml of glacial acetic acid and ۱۴۰ ml of distilled water. So as long as the context is completely transparent, gel and protein strips are revealed.

Gel imaging and statistical analysis

After the end of the Staining process, gels were scanned in a resolution of ۳۰۰ dpi using GS-۸۰۰ scanner (Bio-Rad). Then, their images were analyzed by Image Master ۲.۰ software, identifying spots, determining the amount of protein and a pair of spots has been studied using this software. The molecular weight of the protein spots in the gels was estimated with implementation of simultaneous electrophoresis of standard protein markers. Isoelectric point was determined by the placement of the protein spots on the ۱۳-cm strip with linear pH range ۳-۱۰.

Bioinformatics analysis

The identification of proteins was carried out in accordance with peptide fragmentation methods and by searching in NCBI database using the MASCOT program. The role of proteins has been detected by searching the database of Swiss-port / TrEMBL and using uniprotKB. (<http://uniprot.org>)

Results and discussion

The results showed that from among ۳۶۰ identified proteins, ۳۱ cases showed significant changes. To evaluate the quantitative changes of proteins expression, volume percentage of each spot was used as a normalized value. The data were then analyzed with SAS software. To determine the volume percentage changes of spots in various stages of leaf growth, the F-test was used at a confidence level of ۵%. In this study, each growth stage was investigated with three replicates and a total of ۹ gels. The place of signified spots was shown in the development of leaf growth on the gel in Figure ۱ and all spots with significant spots were presented in Table (۸).

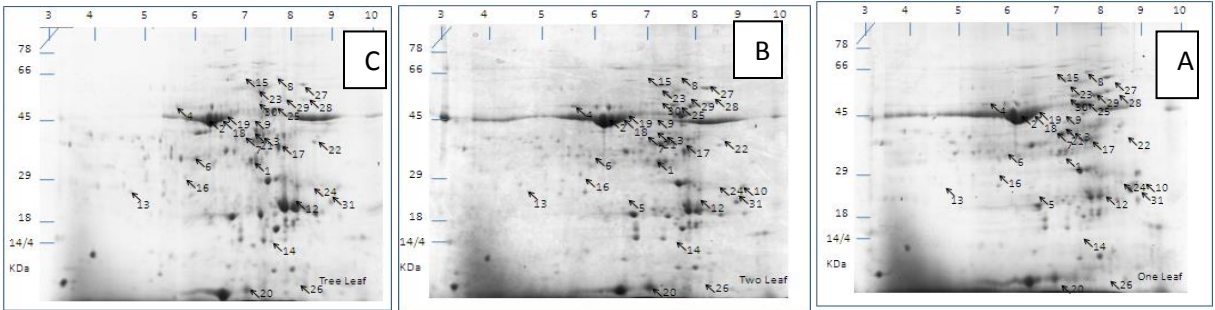


Figure ۱: Location of signified spots in the unifoliate stage (A), bifoliate stage (B), trifoliate stage (C) on the gel

Table ۸: Proteins with significant changes and identified trend in various stages of leaf development (growth)

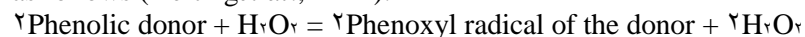
Spot	Proten Name	pI	MW (KDa)	Average changes		
				Unifoliate	Bifoliate	Trifoliate
۱	Cytosolic glyceraldehyde-۳-phosphate dehydrogenase	۷/۶۳	۳۲/۶	۰,۲۳۴	۰,۲۴۹	۰,۰۷۴
۲	Ferredoxin-NADP(H) oxidoreductase	۶/۳۶	۴۳	۰,۳۶۵	۰,۱۴۱	۰,۱۱۹
۳	۳-dehydroquinate dehydratase	۷/۶۱	۳۶	۰,۰۶۰	۰,۰۹۰	۰,۰۳۱
۴	Ribulose-۱,۵-bisphosphate carboxylase activase isoform ۱	۵/۴۸	۴۸/۳	۰,۱۲۰	۰,۱۱۵	۰,۰۰۰
۵	Dehydroascorbatereductase(DHAR)	۶/۳	۲۶/۵	۰,۰۴۳	۰,۱۰۴	۰,۲۹۴
۶	sesquiterpenecyclase	۶	۳۶	۰,۰۶۴	۰,۱۲۸	۰,۳۲۳
۷	PeroxidasePrecursor	۷/۰۱	۳۸/۲	۰,۲۳۸	۰,۱۱۴	۰,۰۷۰
۸	Globulin ۳	۷/۷۸	۶۶	۰,۱۶۸	۰,۱۱۳	۰,۰۶۴

٩	Glyceraldehyde-٣-phosphate dehydrogenase A, chloroplast	٧/٣٤	٤٢/٦١	٠,١٨٣	٠,٠٦٦	٠,٠٤١
١٠	Class II chitinase	٨/٦٦	٢٨	٠,١٦١	٠,٠٤٨	٠,٠٣٧
١١	Thioredoxin M(TRX-M )	٨/٧	١٩	٠,١٣٩	٠,١٣٦	٠,٢٥٧
١٢	Manganese superoxide dismutase	٧/٨٩	٢٥	٠,٢٧٣	٠,٥٩٣	١,٤٠١
١٣	Carbonic anhydrase-like protein	٤/٦٣	٢٧	٠,٠٧٥	٠,٠٣٩	٠,٠٠٩
١٤	High-affinity phosphate transporter PT١	٧/٨٥	٢٠	٠,٠٥٦	٠,١١٦	٠,١٥٠
١٥	Precursor- OxidoreductaseFerredoxin	٧/١٨	٦٤	٠,١٣٣	٠,٠٩٤	٠,١٤٣
١٦	Ferritin	٥/٣٤	٢٧/٥	٠,٢٤٩	٠,١٦٢	٠,١٢٨
١٧	Malate dehydrogenase glyoxysomal precursor	٨/٢٥	٣٥	٠,٠٣٣	٠,٠٤٩	٠,٠١٦
١٨	Ribulosebiphosphate carboxylase/oxygenaseactivase , chloroplast	٥/٩٨	٤٤/٧	١,٤٥٣	٠,٨٦٥	٠,٩٢٥
١٩	Aspartate aminotransferase	٦/٧٧	٤٢	١,٦٦٧	٠,٦٣٣	٠,٤٩٩
٢٠	RuBisCO Small subunit	٧/٠٦	١٥	٢,٣٤٧	١,١٠٥	٠,٩٥١
٢١	Glyceraldehyde-٣-phosphate dehydrogenase	٧/٥٠	٣٩/٥	٠,٠٦٠	٠,١٢٩	٠,٣٦٨
٢٢	ATP synthase subunitgamma	٨/٤٤	٣٩/٨	٠,١١١	٠,١٥٠	٠,٠٦٠
٢٣	Glucose-٦-phosphate ١-dehydrogenase, cytoplasmic isoform ١	٧/٤٣	٥٥/٩	٠,٠٤٤	٠,٠٢٢	٠,٠١٩
٢٤	Cytochrome b٦-f complex iron-sulfur subunit	٨/٤٧	٢٣/٧	٠,٢٩٦	٠,٢٤٧	٠,١٦٠
٢٥	Ribulosebiphosphate carboxylase activase B	٨/١٠	٤٤/٥	٠,١٩٧	٠,١٢٩	٠,٠٧٨
٢٦	Glutelin Precursor	٨/٢٠	١٤/٥٠	٠,١٨٦	٠,٠٨٨	٠,٢٧٠
٢٧	Bowman-Birk type proteinase inhibitor II-٤	٨/٤٥	٥٩/٤١	٠,٣٧٥	٠,٢٩٤	٠,١٦٨
٢٨	Ribulose-١,٥-bisphosphate carboxylase activase-	٨/٦٢	٤٧/١	٠,١٩٧	٠,١٢٧	٠,٠٧٧
٢٩	٢٢ KDa Oxygen-evolving protein of photosystem II	٨/٨٤	٢٧	٠,١٨٩	٠,٠٩٨	٠,٠٣٥
٣٠	Putative fructose ١-,٦ biphosphatealdolase	٧/٧٢	٤٨/٥	٠,١٠٦	٠,٠٧٤	٠,٠٢٢
٣١	unidentified protein	٨/٣٠	٥٠	٠,١٧٤	٠,٠٨٧	٠,٠١٣

Proteins that their expression is reduced with increase of vegetative growth in different stages of leaf growth

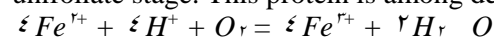
Spot (٧), is probably Ferredoxin-NADP (H) oxidoreductase enzyme (Gaoet al., ٢٠١١). The change trend of this protein is downward, its amount in the bifoliate stage is ٠,٣٨ and in the trifoliate stage is ٠,٣٢ of its value at the unifoliate stage. Ferredoxin-NADP oxidoreductase (FNR) is the last enzyme in the electron transport cycle in photosynthesis from photosystem ١ to NADPH. Then NADPH is used as a reducing reaction in Calvin cycle. Electron cycle is carried out from Ferredoxin to NADPH only in the optical photosynthetic system and the activity in the dark section is prevented. In non-photosynthetic organelles, the FNR acts as reducer of Ferredoxin for several metabolic pathways containing nitrogen fixation, biosynthesis of terpenoids, steroids metabolism and response to oxidative stress (Gummadova, ٢٠٠٣). FNR is a soluble protein, observed as free in the chloroplast stroma and bonded in the thylakoid membrane. (Caruso et al., ٢٠٠٩) observed the reduced expression of this enzyme in response to drought stress of wheat. Spots ٤ and ٢٨ are probably Ribulose ١,٥-bisphosphate carboxylase activase isoform ١ (Gaoet al. ٢٠١١). The change trend of this protein is downward, its amount in the bifoliate stage is ٠,٩٥ and in trifoliate stage is zero of its value in unifoliate stage and the amount of Spot ٢٨ in the bifoliate stage was ٠,٦٤ and in the trifoliate stage is ٠,٣٩ of its value in unifoliate stage. This enzyme was activator of RuBisCO. RuBisCO (Ribulose-١,٥-bisphosphate carboxylase) is a main enzyme in plant photosynthesis. Before catalyzing carboxylation or oxygenation of Ribulose-١,٥-bisphosphate, RuBisCO must be activated by ATP-dependent RuBisCO activating enzyme. In higher plants, there are two forms of RuBisCO-activating proteins with molecular weight of ٤١ and ٤٧ kDa. (Portis, ١٩٩٢; Streusand and Portis, ١٩٨٧). The role of the RuBisCO activation enzyme is to remove Ribulose ١,٥-bisphosphate carboxylase (through ATP hydrolysis by light). It inactively connects to RuBisCO activation site in the darkness and thus releases these sites for activation by CO٢ and Mg٢C (Love and Long, ٢٠٠٠). Spot No. ٧, may be an enzyme called Peroxidase and from Peroxidase family. The change trend of this protein is downward, its amount in the bifoliate stage is ٠,٤٧ and in trifoliate stage is ٠,٢٩ of its value at unifoliate stage (Xiao et al., ٢٠٠٨). The role of this enzyme is removal of H٢O٢, the oxidation of the toxic reducers,

biosynthesis and degradation of lignin, creating cork, catabolism of auxin and responding environmental stresses such as wounding, pathogens attack and oxidative stress. The catalytic activity of this enzyme is as follows (Herting *et al.*, 1991):



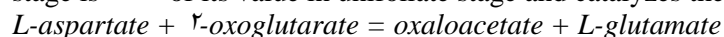
Reduced expression of this enzyme in wheat seedling roots under salt stress has been reported by (Gao *et al.*, 2011).

Spot 1 is probably a protein called Globulin 2 (Wang, 2012). The change trend of this protein is downward, its value in the bifoliate stage is 0.14, and in trifoliate stage is 0.38 of its value at the unifoliate stage. Globulin is a family of globular proteins with more molecular weight than albumin. Globulin is divided to four classes: Alpha 1 Globulin, Alpha 2 Globulin, Beta Globulin and Gamma Globulin (A GROUP of gamma globulins called immunoglobulins are still known as "antibody"). Spot 2, is possibly protein chloroplast glyceraldehyde-3-phosphate dehydrogenase (Maldonado *et al.*, 2008). Change trend of the protein is downward, its amount in the bifoliate stage is 0.36 and in trifoliate stage is 0.22 of its value in unifoliate stage. Chloroplast glyceraldehyde-3-phosphate dehydrogenase leads to accelerate the reduction of the Calvin cycle in higher plants. From among two created subunits of A and B, subunit A contains 335 amino acids and the subunit B contains 368 amino acids. Spot (3), is probably Class II chitinase protein (Wang, 2012). Change trend of this protein is downward, and its amount in the bifoliate stage is 0.29 and in the trifoliate stage is 0.23 of its value at the unifoliate stage. Chitinase is a class of glycoside hydrolase enzymes. Chitinase leads to damage and break of the glycosidic bonds in chitin structure. Chitinase enzyme has mostly molecular mass of about 10 kDa and 43 kDa. Spot No. 16, is probably a protein called Ferritin (Wang, 2012). Change trend of this protein is downward, and its value in the bifoliate stage is 0.60 and in the trifoliate stage is 0.01 of its value at the unifoliate stage. This protein is among detoxifier proteins that catalyzes the following reaction:



Spot (4), is probably a protein called Aspartate aminotransferase (Wang, 2012).

Change trend of the protein is downward, its amount in the bifoliate stage is 0.35 and in the trifoliate stage is 0.30 of its value in unifoliate stage and catalyzes the following reaction:



Spot (5), is probably the RuBisCO Small Subunit (Gao *et al.*, 2011).

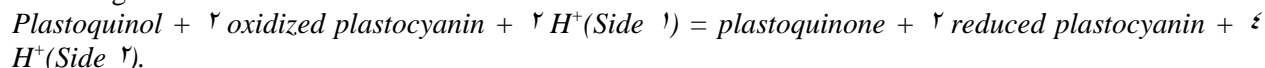
Change trend of this protein is downward, its amount in the bifoliate stage is 0.45 and in the trifoliate stage is 0.4 of its value at the unifoliate stage. Enzyme RuBisCO is one of the key multi-subunit proteins (8 large subunits and 8 small subunits) in carbon fixation pathway and catalyzes the first reaction of the Calvin cycle (Caruso *et al.*, 2008). Small subunits of this enzyme are encoded by the chloroplast genome. RuBisCO is a major source of protein in the leaves and thereby is an Amino acid source for generative components. RuBisCO is involved in aging process of the leaves, the transfer of nutrients from leaves to generative parts which a lot of these metabolites are because of the breakdown of leaf proteins. Environmental stresses can cause reversible or irreversible deactivation of RuBisCO.

Irreversible deactivated RuBisCO is replaced by newly synthesized samples. A clear response in plants during these physiological processes is the development of oxidation processes caused by reactive oxygen species. In accordance with the results observed in this study, decreasing expression of this subunit in durum wheat leaf proteome has also been reported by Portis (Portis, 2003). Spot (23), is possibly a protein called Glucose-6-phosphate 1-dehydrogenase, cytoplasmic isoform 1 (Xia *et al.*, 2008). Change trend of this protein is downward and its amount in the bifoliate stage is 0.00 and in the trifoliate stage is 0.43 of its value in the unifoliate stage. It is a cytosolic enzyme that catalyzes the following chemical reaction:

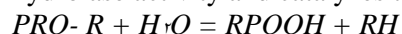


Spot (24), is probably Cytochrome b6-f complex iron-sulfur subunit protein (Hou *et al.*, 2009). Change trend of this protein is downward, and its amount in the bifoliate stage is 0.43 and in the trifoliate stage is 0.04 of its value at the unifoliate stage. Complex cytochrome B6-F is mediate of transferring electron

between photosystems I & II. B<sub>6</sub>/F cytochrome is the complex subunit of Iron-Sulfur that catalyzes the following chemical reaction:



Spot (۳۵), is probably a protein called Ribulosebiphosphate carboxylase activase B. (Donnelly *et al.*, ۲۰۰۵). Change trend of the protein is downward, and its amount in the bifoliate stage is ۰,۶۵ and in trifoliate stage is ۰,۳۹ of its value in unifoliate stage. Spot (۳۷), is possibly Bowman-Birk type proteinase inhibitor II-ε protein. This protein is considered as protease inhibitor proteins (Kamal *et al.*, ۲۰۱۰). Change trend of the protein is downward, and its amount in the bifoliate stage is ۰,۷۸ and in trifoliate stage is ۰,۴۵ of its value at the unifoliate stage. Members of this family have a repeated structure and generally have two separate sites of inhibition. Spot (۳۹), is possibly Oxygen-evolving protein of photosystem II KDa ۲۳ protein. (Wang, ۲۰۱۲) Change trend of this protein is downward and its amount in bifoliate stage is ۰,۵۲ and in the trifoliate stage, it was ۰,۱۸ of its value at the unifoliate stage. The role of this protein is to separate oxygen from water and is involved in photosynthesis and Fixation in photosynthesis II and is found in chloroplast membrane Thylakoid membranes. OEE proteins contain three subunits OEE۲ (۲۳KDa), OEE۱ (۳۳KDa) OEE۳ (۱۶KDa). These proteins are encoded by nuclear genome and are placed at chloroplast membrane luminal surface (Robinson and Klossgen, ۱۹۹۴) Reduced expression of this protein was observed under drought stress in Hajheidari *et al* research conducted on beet. Ford *et al.* (۲۰۱۱) reported the increased expression of four proteins OEC (PSbP, PSbQ, PSbO and PPL homologous of PSbP) under the impact of drought stress in wheat varieties. Spot (۳۰), is possibly Putative fructose ۱-, ۶ biphosphatealdolase protein (Xia *et al.*, ۲۰۰۸). Change trend of this protein is downward, its amount in the bifoliate stage is ۰,۶۹ and in the trifoliate stage is ۰,۲۰ of its value in unifoliate stage. The main role of this enzyme is metabolism of carbohydrates and catalyzing four stages of ten steps of glycolysis. Molecular role of this enzyme is dehydrating and doing phosphorus ester hydrolase activity and catalyzes the following reaction:



This enzyme is involved in metabolism pathway and this decrease can indicate the possibility of reducing expression of proteins in metabolism pathway. In Caruso *et al* studies (۲۰۰۹), decreased expression of this protein is shown in wheat. Spot No. ۳۱, is probably an unidentified protein (Houet *et al.*, ۲۰۰۹). Change trend of this protein is downward, and its amount in bifoliate stage is ۰,۵ in and in trifoliate stage is ۰,۷ of its value at the unifoliate stage.

### Proteins that their expression levels increase with promotion of vegetative growth in different leaf development stages

Spot No. ۵, is possibly Dehydroascorbatereductase (DHAR) enzymes (Lee *et al.*, ۲۰۰۷). Change trend of this protein is upward and its value at bifoliate stage is ۲,۴۱ and in trifoliate stage is ۶,۱۳ times higher than that in unifoliate stage. This enzyme is involved in glutathione-ascorbate cycle in the removal of reactive oxygen species. Among the enzymes studied, dehydroepiandrosteroneascorbatereductase showed the greatest increase during seed development activities. (Bonhomne *et al.*, ۲۰۰۹) witnessed a decrease in the expression of this protein during the drought stress of wheat. Spot (۱۲) is likely a manganese superoxide dismutase protein (Wang, ۲۰۱۲). The change trend of this protein is upward and its amount in bifoliate stage is ۲,۱۷ and in trifoliate stage is ۵,۱۳ times higher than that in unifoliate stage. Superoxide dismutases are Metalloenzymes that their expression changes in different environmental conditions (Bowler *et al.*, ۱۹۹۲) These enzymes, are powerful catalysts that play a major role in antioxidant pathway (Foyer *et al.*, ۱۹۹۴) Isoform of this enzyme was found in the chloroplast. It was observed that this enzyme is active in the plant organelle together with ascorbate peroxidase, monodehydroascorbate Reductase and glutathione reductase. SODs are divided to subgroups of FeSOD, MnSOD and Cu-ZnSOD based on their metal cofactors. Chloroplasts normally have Cu-ZnSOD and ferrous compounds that can be seen in some species (Van Camp, ۱۹۹۴). MnSOD exists in mitochondria and Cu-ZnSOD is found in Cytosol in addition to chloroplast and probably in extracellular space



(Alscheret *et al.*, ۲۰۰۲). In Caruso *et al* studies (۲۰۰۸) decreased expression of this protein is shown on wheat during drought stress. In proteomics research, increase of isoform expression levels of SOD in drought stress (Hajheidari *et al.*, ۲۰۰۵), ozone (Agarwalet *et al.*, ۲۰۰۲) and treated with auxin (Rakwal and Kumatsu, ۲۰۰۶) have been reported. Spot No. ۱۳, is probably carbonic anhydrase-like protein. Change trend of this protein is upward and its value in bifoliate stage is ۲,۶۸ times and in trifoliate stage is ۲,۸۴ times higher than its value in unifoliate stage. This protein is a part of metabolism proteins. (Gaoet *et al.*, ۲۰۱۱) The name of this protein gene is CA۱ and its sequence length is ۲۲۳ amino acids and Gene Sequencing has fully been done for it in rice. Spot (۱۴), is probably high-affinity phosphate transporter PT۱ (Gaoet *et al.*, ۲۰۱۱). The change trend of this protein is upward, its amount in bifoliate stage is ۲,۰۷ and in trifoliate stage is ۲,۶۷ times higher than its expression in unifoliate stage. This protein is found in the plasma membrane and intracellular organelles. This protein as a material-transmitter, acts from one side of the plasma membrane to the other side. The molecular and specific role of this enzyme is transfer of phosphate from one side of membrane to the other side based on a concentration gradient (Donnelly *et al.*, ۲۰۰۵). The expression of this protein increases with increasing severity of drought stress (Mehrabi *et al*, ۲۰۱۳). Spot (۲۱) is probably Glyceraldehyde-۳-phosphate dehydrogenase. The change trend of this protein is upward and its value in bifoliate stage is ۲,۱۵ times and in trifoliate stage is ۶,۱۳ times higher than that in unifoliate stage (Sander *et al* ۲۰۱۱). This enzyme catalyzes the reaction related to NAD (H) in recycling Dihydroxyacetone phosphate (DHAP) or glycerol phosphate to glycerol-۱-phosphate (GIP). GIP plays role in structural skeleton of phospholipids in cellular membrane. This enzyme can also be seen in the cytoplasm. This enzyme is an oxidoreductase and has two biological roles: One in the metabolic processes of Glacier phospholipids and another in the biosynthesis of phospholipids. Accordingly, it catalyzes the following reaction:



With production of Glyceraldehyde ۳-phosphate, Glycolic pathway will be able to begin the recycling stages of usable energy. Glacier aldehyde enzyme ۳-phosphate dehydrogenase catalyzes oxidation reaction of aldehyde from carboxylic acid and releasing energy required to convert +NAD to NADH and Phosphorylation (using inorganic phosphorus) of glyceraldehyde ۳-phosphate (for the production of ۳,۱-Bisphosphoglycerate) catalyzes the (Bustos and Iglesias, ۲۰۰۲). Reduced expression of Glyceraldehyde ۳-phosphate dehydrogenase after treating wheat with a long cold period was reported by Rinalducci *et al.* (۲۰۱۱). Moreover, reducing this protein in response to drought has been reported by MohammadiBazargani *et al.* (۲۰۱۱).

### Proteins that sis not present regular and significant changes in different developmental stages of leaf growth

From a total of ۳۱ detected protein spots, ۸ spots possibly include proteins Cytosolic glyceraldehyde-۳-phosphate dehydrogenas, ۳-dehydroquinate dehydratase, Thioredoxin M (TRX-M), Precursor Ferredoxin-nitrite reductase, glyoxysomal precursor Malate dehydrogenase, ATP synthase subunit gamma , Ribulose-۱,۵-bisphosphate carboxylase / oxygenaseactivase, chloroplast and in various stages of leaf development were changed before tillering, but not significantly and regularly. These proteins are seen in different stages of wheat growth . In Mahammadi *et al* studies (۲۰۱۱). Decreased expression of Cytosolic glyceraldehyde-۳-phosphate dehydrogenas has been shown during drought stress on wheat. In Mohammadi *et al* studies (۲۰۱۱) reduced expression of Thioredoxin M was shown in drought stress on wheat. Reduced expression of Ferredoxin enzyme was reported by Caruso *et al.* (۲۰۰۹) under drought stress. Salekdeh *et al.* (۲۰۰۲) and Hajheidari *et al.* (۲۰۰۵), observed increased expression of Rubisco in rice and sugar beet respectively under drought stress. Rinalducci *et al.* (۲۰۱۱) reported reduced expression of the ATP synthase subunitgamma enzyme due to the effect of heat on winter wheat (Figure ۱).

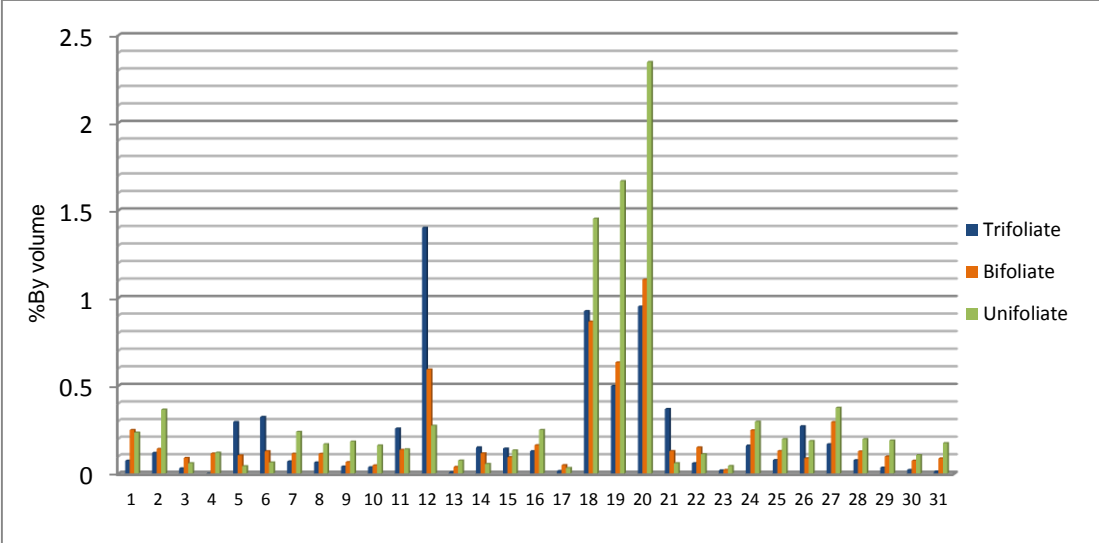


Figure ١- The change trend of ٣١ proteins identified in the developmental stages of leaf growth before tillering

**The role of significant proteins in the stage of leaf growth before tillering**

Proteins were individually discussed concerned with their role and their relationship in different stages of leaf development as follows:

Most of the proteins identified in the leaf growth stage before tillering ٢٢/٢٥ Oxidoreductase, ١٦/١٢٥ Carbohydrate metabolism, ١٢/٩ contributed metabolism

The least changes in response to stress proteins, photosynthetic, protease inhibitor and seed storage proteins, ATP- binding proteins, chaperones, aminotransferases, membrane transport, electron transport, glycosidases and nutrient transport. The percentage of the proteins involved in different stages of leaf development is summarized in Chart (٢).

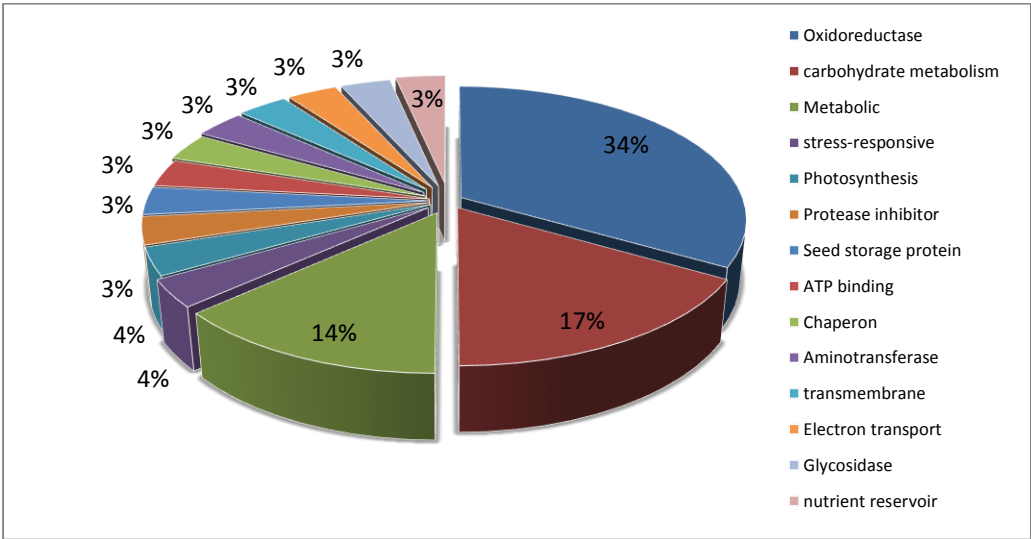


Figure ٢: Percentage of significant changes based on the role and function of them

**Conclusion:**

The volume of data obtained from proteome studies using two-dimensional electrophoresis gel is rapidly increasing. Thus, the interpretation and application of this data is necessary in improving our understanding of biological processes. Proteomics, enjoys desirable potential for use in the reform

processes and paves the way for genetic improvement of plants for reformers with the ability to explore the protein. In order to compare the changes in protein profile of wheat leaf and changes in a wide range of proteins in leaf growth stage before tillering and identifying the proteins involved in progress of this stage, Wheat (Var. Azar ۲) was studied and compared in unifoliate, bifoliate and trifoliate stages. The results showed that a wide range of metabolic activities, including photosynthesis, respiration, proteins biosynthesis, proteins transport, assembly and packaging of proteins, carbohydrates biosynthesis, signal transduction system, detoxification and the basic genetic processes (replication, transcription and translation) underwent change during different stages of leaf growth. In this study, proteins underwent significant changes were divided into three groups:

The first group is proteins that with increasing number of leaves in different stages of leaf development, their value decreases, i.e., the change trend of these proteins is significant and downward so that in the unifoliate stage the greatest value and in trifoliate stage the lowest value are observed. The second group was proteins that their number of leaves increases in various stages of leaf growth, i.e., the change trend of this protein is significant and upward, so that the lowest value is observed in unifoliate stage and the greatest value is observed in trifoliate stage. And the third group is related to proteins that with increasing the number of leaves in various stages of leaf development did not show significant regulatory changes. A more general look reveals that the proteins that showed change in expression at different stages of leaf change, often showed reduced expression of gene, and most of these proteins are chloroplast enzymes and some were mitochondrial enzymes. This can indicate that the increase in the number of leaves reduced expression of some mitochondrial enzymes, which results in an increase in respiration rate of plants. On the other hand, a large number of proteins that underwent reduced expression are chloroplast and in particular photosynthetic enzymes which represent the plant management to reduce its production rate by reducing photosynthesis and avoiding waste of energy. This class of proteins was more kinases and ATPases and protective and regenerative proteins were less found among them. More precisely on how these changes occur, it appears that the proteins with upward change trend in various stages of leaf development, or are considered as regenerative and protective proteins that prevent further damage and damaging activities such as reactive oxygen species (ROS), or are involved in organizing and energy consumption drive the plant to efficient and more economical energy course. The results showed that, the most important identified proteins include ferritin, ascorbate peroxidase and RuBisCOactivase. The aforementioned proteins are all enzymes and involved in the mechanisms of detoxification and removal of oxygen free radicals (peroxidase, ascorbate peroxidase), homeostasis of iron (ferritin) and or activation of other enzymes (RuBisCOactivase). Finally, it can be concluded that the behavior of plants in different vegetative stages is very different and extremely complex and identifying these behaviors, interpretation and understanding the relationships between traits related to vegetative growth of leaf, requires very detailed and diverse testing and integrating the results. For example, if this study be able to determine the characters of all proteins modified in leaf development stage, perhaps more information regarding the relationship between protein and leaf development stage were achieved. However, by increasing our knowledge of the structure and function of proteins and finding closer relationship between the basic components of the cell, more practical applications in knowledge of proteins of bifoliate stage of leaf may be achieved.

# References:

- Agarwal.G.K., R. Rakwal., M.Yonekura and H. Saji.(۲۰۰۲). proteome analysis of differentially displayed proteins as a tool for investigating ozone stress in rice (*Oryza sativa* L.) seedling proteomics, ۲: ۹۴۷-۵۹.
- Alscher.R.G., N. Erturk and L.S. Health.(۲۰۰۲). Role of superoxide dismutase (SODs) in controlling oxidative stress in plants. J. Exp. Botany, ۵۳: ۱۳۳۱-۱۳۴۱.
- Blum, H., Beier, H., Bremer, E. (۱۹۸۷).*Electrophoresis*, ۸, ۹۳- ۹۹.
- Bonhomme. L., R. Monelus., D.Vincent., S. Carpin., S. Claverol., A.M. Lomeneeh., V. Labas., Ch. Plomion., F. Brignolas., D. Morabito. (۲۰۰۹). Genetic variation and drought response euramericans genotypes through ۲-DE proteomic Analysis of leaves from field and glasshouse cultivated plants, phytochem ۷۰, ۹۸۸-۱۰۰۲.

- Bowler. C and M.W. Montagu., D. Inze. (۱۹۹۲). Superoxide dismutase and stress tolerance. Ann. Rev. plant physiol. Plant Mol. Biol., ۴۳: ۸۳-۱۱۶.
- Bradford, M.M. (۱۹۷۹). A rapid and sensitive for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, ۷۲: ۲۴۸-۲۵۴.
- Bustos.D.M and A.A. Iglesias.(۲۰۰۲). Non-phosphorylating glyceraldehyde-۳-phosphate dehydrogenase is post-translationally phosphorylated in heterophic cells of wheat (*Triticumaestivum*L.). FEBS Letters, ۵۳۰: ۱۶۹-۱۷۳.
- Cabello, P., Aguera, E. and Haba, P. (۲۰۰۶) Metabolic changes during natural ageing in sunflower (*Helianthus annuus*) leaves: expression and activity of glutamine synthetase isoforms are regulated differently during senescence. Physiologia Plantarum ۱۲۸: ۱۷۵-۱۸۵.
- Caruso. G., C. cavaliere., C. Guarino., R. Gubbiotti., P. Foglia and A. Lagana. (۲۰۰۸). Identification of change in *Triticum durum* L. leaf proteome in response to salt stress by two-
- Caruso. G., C. cavaliere., P. Foglia., R. Gubbiotti., R. Samperi and A. Lagana. (۲۰۰۹). Analysis of drought responsive proteins in wheat (*triticum durum*) by ۲D-PAGE and MALDI-TOF mass spectrometry. Plant Science, ۱۷۷: ۶۴۱-۶۴۴.
- Damerval, C., M.Zivy., F.Granier. and D.Devienne. (۱۹۸۹). Two-dimentional electrophoresis in plant biology. Adv. Electrophoresis Springer.
- Dertinger, U., Schaza, U. and Schulze, E.D. (۲۰۰۳) Age-dependence of the antioxidative system in tobacco with enhanced glutathione reductase activity or senescence-induced production of cytokinins. Physiologia Plantarum ۱۱۹: ۱۹-۲۹.
- Donnelly. B.E., R.D. Maddan., P. Ayoubi., D.R. Porter and J.W. Dillwith. (۲۰۰۵). The wheat (*Triticumaestivum*L.) leaf proteome. Proteomics, ۵: ۱۶۲۴-۱۶۳۳.
- Farahani, a. (۲۰۰۷). The importance's of setting combine to eliminate the loss of plants during harvest. Promote the management and Operating system of the Central Province.
- Feller, U. and Fischer, A. (۱۹۹۴). Nitrogen metabolism in senescing leaves. Critical Reviews in Plant Science ۱۲: ۲۴۱-۲۷۳.
- Ford.K.L., A. Cassin and A. Bacic. (۲۰۱۱). Quantitative Proteomic analysis of wheat cultivars with differing drought stress tolerance. Frontiers in plant Science, ۲: ۱-۱۱.
- Foyer.C.H and J. Harbinson. (۱۹۹۴). Oxygen metabolism and the regulation of photosynthetic electron transport. In: Foyer CH, Mullineaux PM, editors. (۱۹۹۴). Causes of photooxidative stress and amelioration of defense systems in plants. New York: CRC Press, ۲-۴۲.
- Gao. L., X. Yan., X. Li., G. Guo., Y. Hu., W. Ma., Y. Yan. (۲۰۱۱). Proteome analysis of wheat leaf under salt stress by two-dimensional difference gel electrophoresis (۲D-DIGE). Phytochem ۷۲, ۱۱۸۰-۱۱۹۱.
- Gummadova.J.O. (۲۰۰۳). Two different isoforms of ferredoxin-NADPH oxidoreductase (fnr) in wheat: some aspects of their regulation. Manchester University, Manchester , United Kingdom.
- Hajheidari. M., M. Abdollahian-Noghabi., H. Askari., M. Heidari., S.Y , Sadeghian., E.S, Ober and G.H, Salekdeh. (۲۰۰۵). Proteome analysis of sugar beet leaves under drought stress. Proteomics, ۵(۴): ۹۵۰-۶۰.
- Herting.C., G. Rebmman., J. Bull., F. Mauch and R. Dudler. (۱۹۹۱). Sequence and tissue-specific expression of a putative peroxidase gene from wheat (*Triticumaestivum*L.). Plant Mol. Biol., ۱۶: ۱۷۱-۱۷۴.
- Hou.D.y., H. Xu., G.y. Du., J.t. Lin., Min Duan., Ai-guang. Guo. (۲۰۰۹). Proteomics analysis chloroplast proteins in stage albinism Line of winter wheat (*triticumaestivum*) FA۸۵. BMB reports. ۴۵۰-۴۶۰.
- Kamal. A.H.M., K.H. Kim., K.H. Shin., J.S. Choi., B.K. Baik., H. Tsujimoto., H.Y. Heo., Ch.S. Park., S. Hee Woo. (۲۰۱۰). Abiotic stress responsive proteins of wheat grain determined using proteomics technique. AJCS ۴(۳): ۱۹۶-۲۰۸
- Lee. D.Gi., N. Ahsan., S.H. Lee., K.Y. Kang., J.D. Bahk., In.J. Lee and B.H. Lee. (۲۰۰۷). A proteomic approach in analyzing heat-responsive proteins in rice leaves. Proteomics, ۷, ۳۳۶۹-۳۳۸۳
- Love.R.A and D.M. Long. (۲۰۰۰). wheat ribulosebiphosphate carboxylase activase gene. EMBL/GenBank/DBJ databases.
- Maldonado. M.A., S. Echevarria., S. Jean-Baptiste., M. Hernandez. (۲۰۰۸). Evaluation of three different protocols of protein extraction for Arabidopsis thaliana leaf proteome analysis by Two-dimensional electrophoresis. PROTEOMICS ۷(۱), ۴۶۱-۴۷۲.
- Mehrabi.A.M., A. Mostafaie., A. Majidi Harvan., R. Haghparast and D. Kahrizi. (۲۰۱۳). The comparison of leaf protein patterns between two tolerant and susceptible varieties of wheat under drought stress. EM International. Eco. Enu. Cons. ۱۹ (۴): ۹۳۵-۹۴۶.
- Mohammadi. B.M., E. Sarhadi., A.A. Shahnejat-B., A. Matros., H.P. Mock., M.R. Naghavi., V. Hajihoseini., M. Mardi., M.R. Hajirezaei., F. Moradi., B. Ehdaie., J.W. Meadows., A. Hulford., C.A. Raines and C. Robinson. (۱۹۹۱). Nucleotide sequence of a cDNA clone encoding the precursor of the ۳۳KDa Protein of the Oxygen-evolving Complex from wheat. Plant Mol. Biol., ۱۶: ۱۰۸۵-۱۰۸۷.

- Mostafaie.A.(۲۰۰۲). "Theoretical and practical guide Protein Electrophoresis in Gel.Yadavaran Publishers.  
Noor Mohammadi, Q, E, Lordship type, Kashani, (۱۹۹۶), Agriculture grain, AhvazChamranmartyrUniversity Press.
- Portis.A.R.Jr. (۱۹۹۲). Regulation of ribulose ۱,۵-biphosphate carboxylase/ oxygenase activity. Annu. Rev. plantphysiol plant Mol. Biol., ۴۳: ۴۱۵-۴۳۷.
- Portis.Jr.A.R. (۲۰۰۲).RubiscoactivaseRubisco catalytic chaperone photosynthesis Researach ۷۵۱, ۱۱-۲۷.
- Qiyen. J.H., X. Pan., Q. Pan., Y. Shi., X. Li., G. Zhang., Y.Wang., S. Xie and S. Shen. (۲۰۰۸). Proteomic analysis of wheat hybrid necrosis.Plant Science. ۱۷۵: ۲۹۴-۴۰۱.
- Rakwal.R and S. Kumatsu.(۲۰۰۴). Absciscic acid promoted changes in the protein profiles of rice seedling by Proteomics analysis. Mol. Biol. Rep., ۳۱: ۲۱۷-۲۳۰.
- Rinalducci. s., M.G. Egidi., S. Mahfoozi., S. Jahanbakhsh., G.L. Zolla. (۲۰۱۱). The in fluence of Temperature on plant development in aernalization-requiring winter wheat: A۲-DE based proteomic in vestigation. PROTEMICS ۷۴,۶۴۳-۶۵۹.
- Robinson. C and R.B. Klosgen.(۱۹۹۴). Targeting of proteins into and across the thylakoid membrane: a multitude of meehanisms. Plant Mol. Biol. ۲۶, ۱۵-۲۴.
- Rossignol.M., J.B. Peltier., H. Mock., A. Matros., A. Maldonado and J. Jorrin.(۲۰۰۶). Plant proteome analysis: A ۲۰۰۴-۲۰۰۶ update. Proteomics, ۶: ۵۵۲۹-۵۵۴۸.
- Ruuhola, T., Ossipov, V., Lempa, K. and Haukioja ۱, E. (۲۰۰۳). Amino acids during development of mountain birch leaves.Chemoecology ۱۳: ۹۵-۱۰۱.
- Salekdeh.Gh.H., J. Siopongco., L.J. Wade., B. Ghareyazie and J. Bennett. (۲۰۰۲). A proteomic approach to analyzing drought- and salt- responsiveness in rice. Field Crops Research, ۷۶: ۱۹۹-۲۱۹.
- Sander. I., P. Rozynek., H.P. Rihs., V. Van Kampen., F.T. Chew., W.S. Lee., N. Kotschy-lang., R. marget., T. Bruening and M. Raulf-Heimsoth. (۲۰۱۱). Multiple wheat flour allergens and cross-reactive carbohydrate determinants bind IgE in baker s asthma, Allergy: ۶۶:۱۲۰۸-۱۲۱۵.
- Streusand.V.J and A.R.Jr.Portis.(۱۹۸۷). Rubiscoactivase mediate ATP-dependent activation of ribulosebiphosphate carboxylase. Plant physiol., ۸۵: ۱۵۲-۱۵۴.
- Van Camp. W., M. Van Montagu., D. Inze.(۱۹۹۴). Superoxide dismutases. In: Foyer CH, Mullineaux PM, editors. Causes of photooxidative stress in plants and amelioration of defense systems. Boca Raton, FL: CRC Press, ۳۱۷-۳۴۱.
- Wang. S., P. Ge., C. Ma., L. Gao., X. Li., G. Guo., W. Ma., Y. Yan. (۲۰۱۲). Comparative proteomic analysis of grain development in two spring wheat varieties under drought stress. ORIGINAL PAPER, Anal BioanalChem , ۴۰۲:۱۲۹۷-۱۳۱۳.
- Xiao.S., Ni.Zhongfu., Y. Yao., Y. Zhang., Q. Sun. (۲۰۰۸). Identi W cation of di V erentially expressed proteins between hybrid and parents in wheat (*Triticumaestivum*L.) seedling leaves. ORIGINAL PAPER, TheorAppl Genet ۱۱۸:۲۱۳-۲۲۵.