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Plant Proteomics and Industrial Oil Plants

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ABSTRACT

Proteomics is one of the applied sciences in biology, this century .Using the mass spectrometer device and the development of databases and bioinformatics tools, a fundamental evolution has taken place in molecular biology and new perspectives have been found in agriculture, pharmaceuticals, molecular biology, etc. Two-dimensional gel electrophoresis methods are a popular technique for protein separation because they allow researchers to quantify protein changes on a large scale. Proteomics is a large-scale evaluation of proteins. The term proteomics was produced in 1997 after the introduction of genomics. Although the use of databases and bioinformatics to interpret the results of proteomic findings is being seriously developed. In the study of huge proteomic data, it is very desirable to use the capacity of multivariate statistical methods, due to having many and extensive variables, because these multivariate statistical methods provide the possibility of statistical analysis of several variables, therefore, the use of this statistical technique recently, it has attracted the attention of proteomics scientists. In addition to understanding the tools of proteomics, we are looking for the practical use of proteomics, especially in agriculture, because today the pressure is on plant breeders to provide "smart plant varieties" that are more suitable genotypes with the ability to tolerate biotic and abiotic stress a wider range of climate changes for dealing with the food insecurity of future generations. Therefore, modern plant breeders need precise genetic modification with a gene tracking system for the modified trait. The only caveat in the application of proteomics in biotechnology applications is that genetic modification must be expressed at the protein level. The purpose of this research is to examine the concepts of some main topics in the field of proteomics in simple language, and of course, case studies of proteomics in plants, especially in industrial oilseed plants.

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1. An introduction to proteomics

Based on previous experiences and studies in the field of proteomics, the hypothesis of conducting this study and its summarized items were formed in my mind, and I will describe the related items. After genomics and transcriptomics, proteomics can be considered the next step to study biological systems. In proteomics, the complexity and breadth of research are much more than in genomics, because the genome of an organism stable is enough, while the proteome varies from one cell to another and even from one time to another in the same cell only by changing. One of the environmental conditions in two cells will form different proteomes (Kakaei, 2015). Proteomics is the most accurate technology to check the amount of protein in the cell because it has recently been found that mRNA is not always translated into protein and the amount of translation into protein depends on the transcribed gene and the physiological conditions of that cell. After the completion of the human genome project, the researchers concluded that the cell behavior and all the activities that are carried out in the cell are the responsibility of the proteins (it cannot be predicted from the sequence of their genes) in order to connect the genome with the behavior of the cells. In short, it should be mentioned that the distance between the genome and the molecular mechanism of cell behavior is covered by the proteome.

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1.1. Proteomics tool

Among the various proteomics tools, two-dimensional electrophoresis, mass spectrometry, and bioinformatics technology can be mentioned. It can be said that the first test in protein laboratories was about the placement of protein spots related to proteomes obtained from bacterial culture medium under different treatments (Klose, 2009). Regarding two-dimensional electrophoresis, it should be noted that first the first dimension is run, and then the molecules are run in the second dimension perpendicular to the first dimension to create an electropherogram in the second dimension. In the first dimension of the electrophoresis system, proteins are linearly separated based on their isoelectric point.

In the second dimension, molecules are separated based on their molecular weight. Since two molecules are very unlikely to be similar in these two characteristics (isoelectric point and molecular weight), molecules are separated with a much higher quality in two-dimensional electrophoresis than in one-dimensional electrophoresis. The first dimension of this technique is called the isoelectric focusing (IEF) electrophoresis technique. In this method, a pH gradient is created for the gel, and one side of the gel is more positive than the other side. The second dimension of this technique includes SDS-PAGE in a discontinuous buffer system where proteins are separated according to the difference in molecular weight. The pH ranges are different based on the type of cell or tissue that we want to separate the protein spots in terms of the two mentioned dimensions, and this work is achieved by optimizing (trial and error or the researcher's experience) the experiment (Issaq and Veenstra, 2008).

Principle of Isoelectric Focusing Two proteins with variable isoelectric points migrate in the presence of a pH gradient and electric field until the net electric charge of a protein becomes zero, at which point migration stops (Fig. 1). The general process of proteomics is reported in Fig. 2 by (Yarmush and Jayaraman, 2002).

1.1.1. Mass spectrometer

The mass spectrometer is one of the most important tools in proteomics projects, which measures the ratio of electric charge to the mass of ionized molecules in the gas phase. In 1981, by inventing the ionization method called Fast Atom Bombardment, Barber removed the limitation of using mass spectrometers for biological samples and played a great role in the advancement of this technology. Using a mass spectrometer, one can obtain information about the protein structure, such as the mass of peptides or the sequence of amino acids, and identify the target protein by searching in specific information banks of protein and DNA. Also, this tool can be used to identify the type and location of protein modifications. Obtaining information about protein properties through a mass spectrometer is done during 3 stages of preparation, ionization, and mass analysis of the sample. Therefore the rapid development of mass spectrometers, paralleled by advances in protein chemistry, provides us with new ways to ask biological questions (Cañas *et al.*, 2006; Kakaei, 2015; Wright *et al.*, 2012).

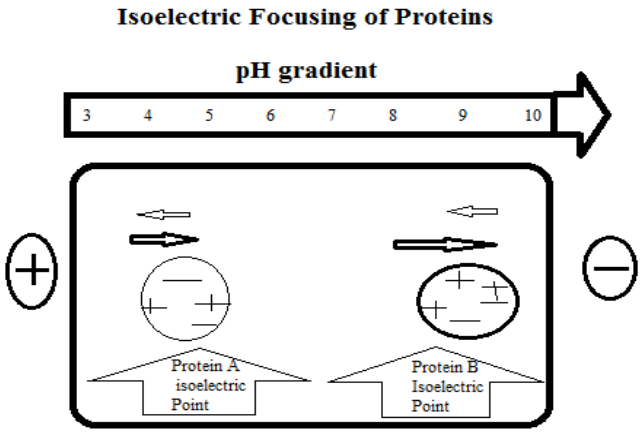

Figure 1. Principle of isoelectric focusing.

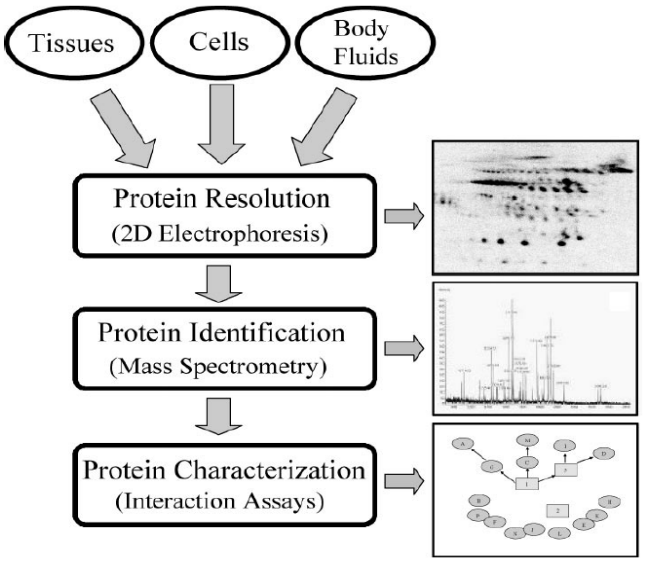

Figure 2. The general process of proteomics (Yarmush and Jayaraman, 2002)

1.2. Different study sections in the science of proteomics

Regarding proteomics techniques, it should be mentioned that in such studies, the desired proteins are identified by their amino acid sequence. Among the most widely used methods are two-dimensional gel electrophoresis (2DE), liquid chromatography/mass spectrometry (LC-MS), selected reaction monitoring (SRM), and surface-assisted laser ionization/desorption (MRM) and protein microarrays (Pandey and Mann, 2000).

1.2.1. Exploratory proteomics

In this part of the study, it should be noted that the main goal of exploratory proteomics is to discover new proteins or discover gene expression patterns in various conditions. Two valuable and relatively new technologies, two-dimensional electrophoresis and mass spectrometry discover a type of proteomics that led to the discovery of new proteins. In this type of study and evaluation, emphasis is placed on proteins whose abundance has changed compared to a reference sample, for example, the presence of a protein in the study sample and its absence in the standard or reference sample indicates the influence of the environmental conditions governing its protein creation. The researcher does not select specific proteins for study, and because new patterns of gene expression are discovered in the proteome, we call it exploratory proteomics (Shirdel *et al.*, 2013).

1.2.2. Target and function-based proteomics

In this field, all the proteins that are identified in the same cell under the same conditions such as growth, differentiation, drug effect, disease, etc., and the possibility of identifying proteins that are expressed in different conditions or changes in their expression are observed, and their performance can be tracked. Of course, identifying these proteins as valuable biological and molecular markers is useful in diagnosing a disease or checking the progress or recovery of that disease. In the plant part, it is possible to subject certain tissues of the same plant to proteomics studies in conditions of moisture stress and conditions without moisture stress and identify the proteins expressed in specific moisture conditions and use that protein as a marker or It is possible to exploit that protein after identifying and ensuring its function in gene production and gene transfer, which tolerates that specific stress in the plant. In other words, the cell produces different proteomes in different environmental conditions. In order to understand the molecular mechanisms of cell behavior and biological reactions, it is necessary to evaluate the proteins that are expressed in a cell, their changes in different conditions, their function, and even the interaction between different proteins in a cell. It is called proteome mapping (Kakaei, 2015).

1.2.3. Comparative and quantitative proteomics

Researchers consider comparative evaluation in some proteomics comparison examines the abundance or quantitative volume of one or more proteins in a proteome is examined compared to the reference proteome. For example, in studies in the field of plant sciences, examining protein and its quantitative and qualitative study, measuring changes in protein expression, determining the position and identifying cellular function at the level of bean proteins in relation to the nutritional stress of the tartan mite using the technique Two-dimensional electrophoresis has been reported (Kakaei, 2017).

1.3. Protein microarray technology (protein chip or protein microarray technology) and quantitative measurements

In this technology Protein array is a valuable tool to identify proteins. Protein arrays allow the study of thousands of interactions simultaneously. The primary and conventional methods of proteome analysis are two-dimensional electrophoresis and mass spectrometry, but despite the effectiveness of these methods, we face the limitation of not recording proteins that have low abundance, so they are not very suitable for diagnostic work because usually proteins with low abundance are desired for detection. Therefore, a more comprehensive method such as a protein array is needed. Currently, microarray technology is a desirable technology in the proteomics approach (Angenendt *et al.*, 2002). Considering the great advantages of this method in the fields of proteomics and even genomics, a significant perspective can be given to this technology.

Although the use of databases and bioinformatics to interpret the results of proteomic findings is being seriously developed. In the study of huge proteomic data, it is very desirable to use the capacity of

multivariate statistical methods, due to having many and extensive variables, because these multivariate statistical methods provide the possibility of statistical analysis of several variables, therefore, the use of this statistical technique Recently, it has attracted the attention of proteomics scientists (Zali *et al.*, 2013).

Multivariate statistical analysis and modeling of data from 2D electrophoresis for comparison and classification is an alternative approach using the combination of all proteins/spots in the gel.

Sometimes in exploratory proteomics, we come across a large space, for example, 50 variables, which is very difficult to investigate the relationships between them, so we use multivariate statistical methods to reduce the number of dimensions in the investigated space. Each of the gel analysis software pursues a specific goal, for example, obtaining biomarkers, or matching points in gels in similar samples to determine the proteomic diversity between two sensitive and tolerant samples to a specific stress. Decomposition into principal components (bi-plot analysis), cluster analysis (clustering into protein categories based on the desired criteria), detection function analysis (confirming the correctness of clustering), correlation analysis (studying the relationships between the expression profiles of protein points), etc. There are multivariate statistical methods that are used in the analysis of two-dimensional gels in proteomic studies. The use and importance of using multivariate statistical methods in proteomic studies have been emphasized and used by many researchers (Zali *et al.*, 2013; Jensen *et al.*, 2008; Jessen *et al.*, 2002; Fullaondo *et al.*, 2001; Kakaei, 2015).

1.4. Protein engineering

Protein engineering is one of the most important branches in the science of biotechnology, which has various sub-branches. According to the definition of protein engineering and protein biotechnology, it is the production and extraction of proteins from animal, plant, and microbial sources and their use in a special biological event. A group of proteins is produced and used in industry, a group for a therapeutic and diagnostic procedure, and another group to make special industrial tools. Choosing the source of protein is the first step in the production process of any type of protein. Plant and animal tissues and microorganisms are among the sources of protein extraction. Plants are also considered one of the important sources of industrial proteins. Proteins can be mass-produced by cultivating animal, plant, and microbial cells (Jackman and Yada, 2009).

1.4.1. Protein synthesis in the living environment

A protein engineer should pay attention to the following three points in order to be able to design and purify a protein:

I. Knowledge of the basic spatial arrangement information of natural protein,

II. Choosing the right organism, until the desired protein gene is expressed in it and it can be obtained after purification.

III. The protein engineer must know the differences between the gene expression mechanism in prokaryotic and eukaryotic systems in order to be able to choose the most appropriate system (Moosavi-Movahedi *et al.*, 2009).

1.5. Case studies in different plants in the field of agricultural proteomics

Kakaei et al. (2017) in a study titled alfalfa plant protein changes under normal conditions and under stress resulting from alfalfa leaf weevil feeding, stated that the use of multivariate statistical methods such as principal component analysis, cluster analysis, and detection function analysis in it is very useful to study protein spots in alfalfa samples under the biological stress of the weevil pest and the reference sample (under control and free from the presence of the alfalfa leaf weevil).

Kakaei (2017) in a study entitled Response of Common Bean proteome to Two-Spotted Spider Mite *Tetranychus urticae* Koch using Proteomics Techniques, stated that proteins play a very important role in the response of plants to biological stress because they play a role in the structural changes and metabolic changes of the plant. Primary metabolites also affect insect preference and performance in plants (Berenbaum, 1995). Evaluation of the expression of proteins with the help of two-dimensional electrophoresis showed that, in general, proteins have a change in expression against the nutritional stress of the two-spotted tartan mite. The presence of most of the proteins expressed in the present study in the group of stress-responsive proteins probably indicates the effect

of these primary metabolites on the preference and performance of insects and their defensive role.

Kakeai and Kiani (2019) in a study titled Evaluation of Proteins Expression Differences of Royan Axes in the Two Cultivars of Rice with Two-dimensional Electrophoresis, stated that the variation in the embryo axis protein pattern in two rice cultivars tolerant and sensitive to salinity (Houssine and Seng Jo cultivar, respectively) was identified and also by using two-dimensional electrophoresis studies, it is possible to easily investigate the significant expression changes due to the difference of cultivars at the embryo proteome level with the help of relevant software and statistical analyzes and determine the index of changes. They also stated that the decrease in the expression of spots in the salt-sensitive variety (Sang Jo) indicates the destruction of proteins.

Kakaei (2015) in the study of alfalfa populations, stated that alfalfa is an important fodder plant and alfalfa leaf weevil is the primary pest of this plant, a research was conducted to investigate the general pattern of expression. The protein content of the leaves of this plant in response to the biological and nutritional stress of the alfalfa leaf weevil pest was determined using the proteomics technique. In order to investigate the effect of stress resulting from alfalfa leaf weevil feeding on alfalfa leaf proteome pattern, a sample of nutritional stress and no nutritional stress under the same growth conditions were obtained to determine the significant difference in gene expression between the t-test method was used for control and stress conditions. The results obtained from the mass spectrometer led to the identification of the desired proteins.

Kakaei (2018) in the study of the application of two-dimensional electrophoresis in identifying the sources of resistance and susceptibility to yellow rust disease in bread wheat, stated that based on the relative resistance indices, susceptible, resistant and semi-resistant cultivars can be identified. Resistant and sensitive were identified among the studied cultivars. Therefore, after examining the protein pattern of these two cultivars, 17 spots had increased expression and 10 spots had decreased expression. Based on the obtained results, the difference in the proteome pattern of two resistant and sensitive wheat cultivars can probably show the change in the expression level of enzymes and proteins involved in resistance. Fig. 3 and Fig.4 show images of a leaf sample in the Rejaw variety of bread wheat in the range of pH 4 to 7 is linear and the Proteome pattern of alfalfa leaf tissue genotype Hamadani in the spotted alfalfa aphid feeding stage.

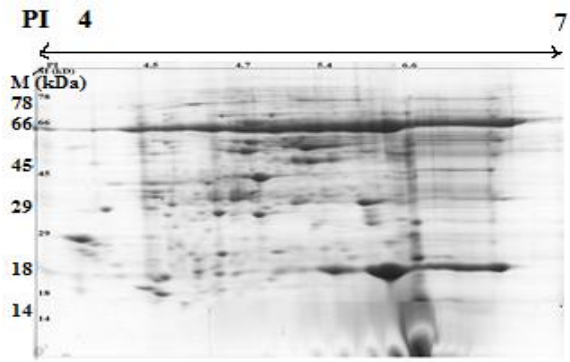

Figure 3. The image of a leaf sample in Rejaw variety of bread wheat in the range of pH 4 to 7 is linear - photo by Mehdi Kakaei

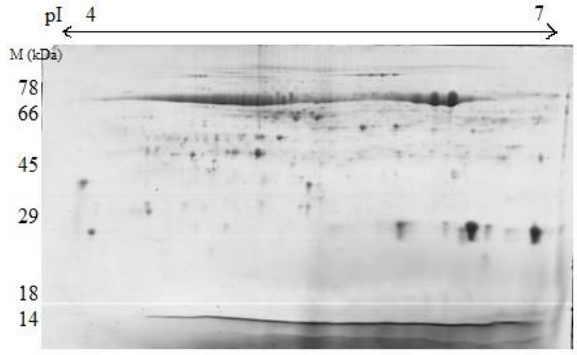

Figure 4. Proteome pattern of alfalfa leaf tissue genotype Hamadani in the spotted alfalfa aphid feeding stage - photo by Mehdi Kakaei

1.6. Case studies about industrial plants and the importance of proteomics

The Camelina plant (*Camelina sativa*), a member of the Brassicaceae family, has been proposed as one of the plants suitable for industrial oilseeds (Neumann *et al.*, 2021). Due to the importance of industrial plants, especially oilseed industrial plants, I will discuss some of the research records in this field below. *Camelina sativa* L. a closely related species of Arabidopsis, is an oilseed crop that is emerging as an important biofuel source. Camelina genome and transcriptome maps have recently become available, but their proteome composition remains unknown. An LC-based quantitative proteomics study was used to characterize the Camelina seed proteome, resulting in the identification of 1532 proteins (Alvarez *et al.*, 2015).

Balbuena et al. (2011) studied sunflower genotypes with a proteomics approach in relation to cold and stated that cold acclimation is a phenomenon in which plants are exposed to low but non-freezing

temperatures before being exposed to extreme temperatures. To investigate how the metabolism of sunflower plants is regulated during low temperatures, they adopted a comparative proteomic approach to identify differentially expressed proteins in frost-sensitive and tolerant leaves. Cold-responsive proteins were more involved in metabolism, protein synthesis, energy, and defense processes in all studied sunflower lines.

Çulha Erdal et al. (2021) in a proteomic study of safflower drought-sensitive and drought-tolerant cultivars, stated that drought is one of the most important environmental stresses that negatively affects the growth and development of oilseeds. Regarding the safflower plant, there is limited knowledge about its proteomic studies. They also stated that a total of 72 protein spots were observed differently the identified proteins were mainly involved in photosynthesis and carbohydrate, defense protein and energy metabolism

López-pedrouso et al. (2014) in a study of common beans say that using the two-dimensional electrophoresis technique, they identified clusters with the highest levels of methionine content in beans, methionine is an essential amino acid that is deficient in bean seeds. Their findings showed that this technique could be used in the genetic improvement of beans.

2. Conclusion

According to the researches and studies that I have done in the field of proteomics and also according to the review of various articles that I have done in this review article, the following summarized results can be reached, which I will state below.

Proteome can be introduced as all the proteins expressed by the genome, a cell, or an organism, and the science of proteomics includes extensive studies such as the isolation of proteins from a sample and the comparison of protein expression in various samples such as the control sample with the sample under treatment or under conditions. Includes special the science of proteomics with its various tools has revolutionized the fields of agriculture, medicine, pharmaceuticals, etc. Although the genome data obtained from genomics is very valuable, it is incapable of answering all biological questions, and post-translational changes cannot be interpreted with genomic studies, so proteomics is a useful tool for describing molecules and biological pathways. In the field of agriculture (in relation to biotic and non-biotic stresses) in order to identify proteins and finally to identify the gene or genes responsible for tolerance to the aforementioned stresses in order to create tolerant plant varieties or species, extensive research has been done in cooperation with genetic engineering science. Some of them have been mentioned. Among other methods of protein separation, the two-dimensional electrophoresis method has attracted the attention of researchers due to the possibility of high reproducibility. The world of proteomics is an infinite world that we are now at the entrance of. Scientists are looking for the application of genome information in the field of proteomics.

In general, in industrial plants such as camellia, sunflower, safflower, canola, soybean, cotton, sugar beet, sugarcane, other medicinal plants, etc., more proteomic studies have been conducted or are being conducted in relation to biotic and abiotic stresses, but with Paying attention to protein changes in different environmental conditions and high environmental variations and high genotypic variations in different plant species, protein studies may not be able to give a specific number of years to complete the proteome project, and most of the proteins identified in the mentioned plants are under stress conditions. Both biological and non-biological are somewhat common. According to the case examples that were mentioned, the valuable and wide world of proteomics science became more visible, and the space for trying to understand the different aspects of this science in agriculture, medicine, etc. is very ready (Kakaei, 2015).

In addition to understanding the tools of proteomics, we are looking for the practical use of proteomics, especially in agriculture, because today the pressure is on plant breeders to provide "smart crop varieties" that are more suitable genotypes with the ability to tolerate a wider range of climate changes for Dealing with the food insecurity of future generations. Therefore, modern plant breeders need precise genetic modification with a gene tracking system for the modified trait. The only caveat in the application of proteomics in biotechnology applications is that genetic modification must be expressed at the protein level.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed Consent

The authors declare not to use any patients in this research.

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