

# Proteomics and its Implication in Modern Crop Breeding

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## Abstract

Plant proteomics is the study of proteins' relationships, functions, compositions, and architectures, as well as their biological functions. It gives a greater grasp of the organism's structure and function than genomics. An organism's or system's proteome is the total set of proteins it produces or modifies. Proteomics allows an ever-increasing number of proteins to be identified. This changes with time and in response to different demands or pressures that a cell or organism faces. This method can be used to determine protein changes during plant development, including somaclonal variation. Although far more difficult than genomics, proteomics is one of the most important methodologies for understanding gene function. Proteomics has a large and diverse range of applications. Many functional genomics techniques, such as microarray-based expression profiles and systematic phenotypic profiles at the organism level, benefit from proteomics. The integration of these proteomics data with bioinformatics methodologies will expose the functional aspects of the genes, which will finally represent protein properties and activities, allowing researchers and plant breeders to fully comprehend the genetic trait of interest. Plant proteomics is so critical in today's crop development effort. Proteomic approaches can be used to study biotic and abiotic stress tolerance in different germplasm or cultivars, as well as to assess small changes in protein expression levels in response to selective breeding. The goal of this review is to assess proteomic research, types, and applications in crop production in general.

**Keywords:** proteomics; crop breeding; yield; technology

## Introduction

Plant breeding methods have changed as a result of technological advances in traditional methods of plant breeding (Moose and Mama, 2008). Plant growth and development are limited by abiotic and biotic stresses, resulting in lower agricultural yields (Gull et al., 2019). Modern technology for genetic sequencing, microarray testing, gene expression and protein within the cell membrane, and cell marking information has been extremely helpful in identifying chromosome regions that cause genetic variation, thus providing far-reaching tools for more selective genetic selection processes (Grover and Sharma, 2016). Proteomics is a comprehensive study of the function and structure of proteins in order to comprehend the nature of an organism (Müller et al., 2020).

Proteomics is a rapidly growing discipline of molecular biology concerned with the systematic, high-throughput analysis of protein expression in cells and organisms. It is large-scale research of proteins (Gillet et al., 2016). Proteins play a range of activities in living organisms, including building muscle fibers, accelerating food digestion, and creating and reproducing DNA. Other types of proteins include antibodies, which protect an organism from infection, and hormones, which transmit vital information throughout the body (Hall and Hall, 2020). Proteomics investigations frequently result in inventories of the protein composition of differently expressed proteins across many situations (Boersema et al., 2015).

Proteomics is becoming increasingly popular in cellular, genetic, and physiological research (Evans, 2015.). Advances in genomics and proteomic technologies have benefited crop breeding programs' success over the last two decades, and will help make substantial progress toward variety development in the near future (Ashraf, 2010). Combining proteomics, genomics, and bioinformatics will improve our understanding of biological systems' data. The study of the proteome, or the protein complement of the genome, is known as proteomics (Cho, 2007). Every year, millions of genes are discovered, many of which encode proteins with unknown functions. The term proteome was used by scientists to describe the entire protein complement encoded by an organism's DNA (Fenstermacher, 2005). To provide a more full understanding of biochemistry at the cellular and organismal level, proteomics is supplementing work on cellular intermediate metabolism and nucleic acid metabolism (Rao et al., 2017).

Proteomics is the study of the entire protein content of a cell, tissue, or organism using technology. In establishing the identity of an organism's proteins and understanding their structure and activity, it works in tandem with other "omics" technologies such as genomics and transcriptomics (Lu and Zhan, 2018). Early disease diagnosis, prognosis, and disease progression monitoring are all dependent on proteomics. As a target molecule, it is also important in drug development. The study of the proteome at any stage, including its expression, structure, functions, interactions, and changes, is

referred to as proteomics (Sundar and Singh, 2018). The proteome evolves over time, between cells, and in response to environmental factors. Proteomics in eukaryotic cells is complicated by post-translational modifications, which can occur in a number of places and in different ways (Aslam et al., 2017).

Plant breeders can benefit from proteomic research combined with functional genomics skills to better understand cellular processes and overcome some of the limitations of traditional crop breeding (Das et al., 2015). Proteomic investigations of a target crop offer a fresh way of finding the genes that are important for crop breeding. Over the last two decades, technological advances in the extraction, separation, quantification, and identification of plant proteins have enabled high-throughput study of crop proteins, and the reproducibility of these technologies has reduced errors in proteomic studies (Tan et al., 2017). Generally, the goal of this review is to assess proteomic research, types, and its implication in crop breeding technologies.

## Types of proteomics

### 2.1 Expression proteomics

The quantitative examination of protein expression between samples that differ in some way is known as expression proteomics (Mayers et al., 2017). This method can be used to compare the protein expression of the entire proteome or subproteomes between samples. This could help researchers find disease-specific proteins. To examine if there are any variations in protein expression, tumor samples from cancer patients and equivalent tissue samples from healthy people might be compared. Two-dimensional gel electrophoresis and mass spectrometry can be used to identify proteins that are over or under expressed in cancer patients compared to healthy people. This might be compared to the microarray data. These findings could help researchers better comprehend tumor growth (Maes et al., 2015).

### Structural proteomics

Unlike expression proteomics, which compares the structure and type of protein complexes found in a biological organelle in a healthy and diseased condition, structural proteomics tries to map out the structure and type of protein complexes found in a biological organelle (Singh et al., 2019). The goal is to identify all of the proteins in a complex and to describe all of their protein-protein interactions. Purification of individual subcellular organelles or protein complexes can help researchers piece together information about cell architecture and explain how the expression of specific proteins confers distinct features on cells (Christopher et al., 2021).

### 2.3 Functional proteomics

Functional proteomics is a broad term that refers to a wide range of targeted proteomics techniques. It is the study of the properties of molecular networks in living cells using proteomics methods (Larance and Lamond, 2015). Finding molecules involved in these networks is one of the key objectives. One of functional proteomics' achievements is the identification and investigation of protein networks involved in the nuclear pore complex. New proteins were discovered as a result of this finding, which aid in the movement of important molecules from the cytoplasm to the nucleus and vice versa (Chanukuppa et al., 2019). The term "functional proteomics" applies to a number of tailored proteomics approaches. Protein-protein interactions are studied to determine protein functions and to demonstrate how proteins form larger complexes. Certain subproteomes are sometimes isolated using affinity chromatography for further investigation (Figeys, 2003).

## Proteomics and their applications in plant breeding

Plant signaling and metabolic processes are two areas where proteomics is becoming increasingly important. Protein-level research is becoming increasingly significant in uncovering molecular pathways that are vital for plant growth, development, and interactions with the environment (Das et al., 2015). Thanks to contemporary proteomic technologies, high-throughput proteome examinations followed by protein identification to study a variety of functional properties, including quantification, protein-protein interactions, and subcellular localization, are now possible (Chen and

Harmon 2006). A single protein diagnostic technique will not give a complete analysis of the entire proteome because the plant proteome is diverse and dynamic. To improve proteome resolution, coverage, and knowledge, researchers use a mix of methods (Mergner and Kuster, 2022).

Crop breeding aims to improve overall crop quality, reduce yield loss, improve disease resistance, and, most importantly, protect crops from various biotic and abiotic stresses (Das et al., 2015). The use of proteomics to precisely assess crop plants, notably in crop breeding, has expanded considerably in the last decade. Proteomics is more advantageous than other -omics methods since a part of the plant proteome provides a full picture of cellular proteins (Komatsu et al., 2013).

Many functional genomics methodologies benefit from proteomics, including microarray-based expression profiles and systematic phenotypic profiles at the organism level. When this data are combined with bioinformatics tools, the functional components of the genes are revealed, which then represent the protein characteristics and functions, allowing researchers and plant breeders to completely appreciate the genetic trait of interest (Hamid et al., 2009).

### 3.1 Improvement of Crop Yields and Quality

Proteomic research aids in the development of high-yielding stress-tolerant lines or cultivars using marker-assisted genetic augmentation on crops (Mustafa and Komatsu, 2021). Proteomics has led to the discovery of numerous proteins involved in plant growth and development (Chen and Harmon, 2006). From an agricultural standpoint, seed viability is one of the most important factors impacting crop productivity. Plant breeders and biologists can examine genes responsible for seed quality and build prediction ideas using proteomic analysis of seed protein content and the roles of enzymes in the starch production pathway (Baslam and Mitsui, 2020; Komatsu et al. 2013).

Heterosis has been the most frequently employed crop breeding technology to date, especially in crop improvement programs that require a sterile male line for hybrid breeding (Khan et al., 2015). Plant breeders may benefit from the use of crop proteomic studies to uncover proteins involved in the regulation of male sterility. Transgenic approaches are becoming an increasingly essential strategy for producing germplasm with desired gene expression in compared to traditional breeding. However, in order to evaluate these genetically engineered crops, a range of proteomic investigations are required to confirm protein expression at desirable levels (Das et al., 2015). Thanks to recent breakthroughs in proteomic technologies, plant breeders and researchers can now overcome challenges in the generation of male sterile lines, and heterosis is being reexamined utilizing proteomics-based, systems-level methodologies to understand underlying molecular processes (Parray et al., 2019).

### 3.2 Combating Abiotic Stresses

In agricultural crop growth and yield, stress is a crucial limiting factor. Seed germination is delayed, plant growth is inhibited, and agricultural yields are reduced as a result of stress (Fahad et al., 2017). Proteins associated with the primary function of an organ, tissue, or organelle accumulate preferentially in that organ, tissue, or organelle. Furthermore, due to the nature of abiotic stress, intracellular compartments dominate plant stress responses (Komatsu et al., 2013). Plants are constantly forced to observe and adjust to detrimental environmental changes in order to avoid unwanted implications on their growth and development (Ahuja et al., 2010).

Plant breeders want to know how agricultural plants resist abiotic stress so that they can develop agriculture breeding strategies that ensure long-term crop production efficiency (Gilliam et al., 2017). Temperature, osmotic balance, light, water supply, and nutritional levels are all affected by abiotic stress on plants. Stress-related modifications in plant development are increasingly causing concern among agricultural plant breeders. Plant breeders can contribute to meeting the demand for more agricultural production by improving plant health in a wider range of adverse environmental conditions (Das et al., 2015). Proteomics allows for detailed protein analysis of stressed plants (Ghosh and Xu, 2014).

### 3.2.1 Heat (High Temperature) Stress

High temperature (HT) is a major abiotic stress that affects plant development, metabolism, and agricultural output all over the world (Dhankher and Foyer, 2018). Heat stress destroys cell membranes and disrupts metabolic processes. In order to survive in such harsh conditions, plants activate various defence systems in response to heat stress (Timperio et al., 2008). To secure the crop plant's survival in such harsh temperatures, plant breeders must create a good protective strategy. Multiple genes, proteins, ion transporters, osmoprotectants, antioxidants, and other factors control plant heat stress tolerance mechanisms, which involve numerous physiological and biochemical changes in the cell, such as changes in plasma membrane structure and function, protein composition, and primary and secondary metabolites (Jha et al., 2014).

Proteomic investigations can help to reveal potential links between protein abundances and plant stress acclimation (Kosová et al. 2011). Proteins play a direct part in heat stress response. In the context of global warming, comprehensive proteome profiling programs are important tools for bettering our understanding of heat stress responses and increasing the breeding of heat-tolerant crops (Das et al., 2015). The discovery of heat-responsive proteins is an important first step toward understanding the molecular mechanisms that will lead to the development of heat-tolerant cultivars that are less susceptible to heat stress (Bita and Gerats, 2013). Plant breeders are faced with the difficult issue of both improving agricultural production and plant survival (Acquaah, 2009).

### 3.2.2 Salinity Stress

Salinity stress is one of the most common types of abiotic stress in plant agriculture, and it has a negative impact on crop output around the world (Shrivastava and Kumar, 2015). Scientists are concentrating their efforts on the development of salt-tolerant plants because salinity stress makes meeting food production targets harder (Ashraf, et al., 2008). Crop breeding has been used to create salt resistant lines for a long time, but it has had limited success due to the multi-genic nature of salt resistance and the lack of genetic variability in major crop plants (Ashraf and Munns, 2022). A proteomic analysis properly confirmed the functional features of individual proteins involved in the salt-tolerant response, giving plant breeders new insights into the salt stress response in plant roots. Molecular networks necessary for salt stress response and tolerance can be established by combining proteomics findings with those from other -omics and bioinformatics approaches (Mokrani et al., 2020).

Proteomic studies revealed functional properties such as photosynthesis, signal transduction, osmotic homeostasis, ROS scavenging system, ion homeostasis, cross-membrane transport, cytoskeleton dynamics, protein destiny, and cross-tolerance to various stimuli (Ditta, 2013). Plant biology, crop breeding, plant disease, and stress biology are all being studied using proteomic approaches right now. Many proteomic approaches have been utilized to investigate the molecular basis of the salt stress response. A proteomic method was recently used to identify the salt stress-responsive proteins in an elite wheat cultivar (Das et al., 2015).

### 3.2.3 Drought Stress

The first step in creating drought-resistant genotypes for crops is to research how they react to drought (Mir et al., 2012). Drought stress is one of agriculture's most basic constraints, in which a lack of water for crop irrigation reduces carbon fixation by the photosynthetic system, resulting in net output losses (Farooq et al., 2012). Plant breeders need unique and more precise approaches to fast-track breeding programs required for the increasing needs of food and fodder due to a changing climate in which drought stress has major international implications on crop damages (Das et al., 2015). Crop loss due to drought has a huge impact on the agriculture industry and the economy. As a result, traditional breeding and/or genetic engineering are being employed to prevent economic losses and to make crop plants more drought tolerant (so that crops can thrive even in drought-prone places) (Malhi et al., 2021).

Some of these proteins may have physiological benefits during drought, making them good candidates for crop breeding selection. Bean breeders have benefited from this understanding of the drought stress response (Cai, 2008). Although plant breeders have made significant advances in plants by developing drought-tolerant lines/cultivars of a number of crops through traditional breeding, this technique has numerous downsides, such being time-consuming, labor-intensive, and inefficient (Choudhary et al., 2019). As a result, plant breeders have benefited from recent proteomic research findings that may soon enhance the traditional breeding method (Eldakak et al., 2013).

### 3.2.4 Cold (Chilling) Stress

The global population is increasing at an alarming rate, but crop production is struggling to keep up due to a variety of environmental challenges. Cold stress (low temperature, 1–10 °C) is a major worry for plant breeders because it threatens the long-term viability of crop yields in specific locations (Shah and Wu, 2019). Plant growth and development are disrupted by cold stress, which results in leaf yellowing (chlorosis), poor germination, limited leaf expansion, stunted seedlings, wilting, and necrosis, among other phenotypic symptoms (Hasanuzzaman et al., 2019).

Most plants can achieve moderate freezing tolerance with cold acclimation. One of the most essential strategies for plants to adapt to freezing is changing plasma membrane compositions and functions (Sadura and Janeczko, 2021). Proteome research is used by plant breeders to investigate these tolerance mechanisms and learn about the various forms of cold stress responses. Comparative proteomics has been utilized to identify the overall response of cold resistant plants as well as their recovery pathways after chilling stress (Mustafa and Komatsu, 2021). As a result, collecting proteomic data in response to cold stress is crucial for crop breeding efforts, with the goal of improving cold resistance in crops (Raza et al., 2021).

This helps researchers better understand how plants adapt to difficult environments and how agricultural production in such a severe environment can be increased. Certain plasma membrane protein expressions have been shown to vary in response to a chilling environment, resulting in an increase in a plant's cold tolerance (Kumar et al., 2020). Current molecular breeding research can be more productive thanks to proteomic discoveries, which add to our understanding of these long-term responses to cold stress, which could be crucial for cold stress adaptation under inescapable temperature extremes. As a result, plant breeders are increasingly using comparative proteomics to better understand cold stress and plant chilling resistance.

### 3.2.5 Flooding Stress

Flooding is another major abiotic stress that has an annual impact on agriculture and crop yield in many natural and artificial ecosystems around the world (Raza et al., 2019). Soil inundation produces a number of physiological problems in plants, resulting in reduced growth and survival. One of the most significant effects of floods is a reduction in metabolism, as well as a reduction in water and nutrient intake. As a result, continual soil flooding will eventually lead to anoxic conditions, which will harm plant respiratory metabolism (Zahra, et al., 2021). Plant breeders are currently having a hard time generating flood-tolerant germplasm to reduce crop loss due to flooding. Understanding genetics and molecular biology can help breeders design flood-tolerant cultivars. Breeders are using quantitative proteomics to generate flood-tolerant plants and boost agricultural productivity (Satya and Sarkar, 2018).

## 3.3 Combating Biotic Stresses

Plant diseases (e.g., fungi, bacteria, viruses, and nematodes) have emerged as one of the most significant roadblocks to boosting crop output and productivity around the world (Hussain et al., 2020). Plant diseases can injure a huge number of crops, with symptoms ranging from mild to epidemic, and result in the devastation of food crops grown across large areas (Jones, 2021). Each year, plant disease causes at least 10% of global food production to be lost. Infections interact with other biotic and abiotic variables, as well as one another, can reduce yields more than expected (Mesterházy et al., 2020). Because pathogenic organisms pose such a threat



to current agriculture, plant breeders are working to develop sufficient methodologies for studying pathogenic detriments and using proteome research to battle them, as well as to develop disease-resistant crops to reduce production losses.

Crop losses from numerous viral diseases have been greatly decreased as a result of combining traditional breeding methods with proteomic techniques (Panis et al., 2020). Thus, the breeders' goal of using proteome analysis to investigate defense responses and applying this knowledge to trait design should benefit in the development of disease resistant crop varieties. Many of the proteins found were associated to disease resistance in plants, showing that defense proteins were activated quickly after inoculation (Hamany Djandee et al., 2020). Incorporating proteomic research on pathogens produced in vitro into crop breeding has proven to be a useful pathological analysis tool (Manikandan et al., 2018). As a result, proteomics has developed into a powerful tool for providing vital information regarding pathogenic and virulent factors, opening up new avenues for crop disease diagnosis and pathogen protection for plant breeders (Belete and Boyraz, 2019).

## Conclusions and Prospects

The recent integration of advances in proteomics, genomics, and molecular marker applications with traditional crop breeding approaches has shaped the foundation for molecular crop breeding and is revolutionizing these century crop improvement programs for improved yields, nutritional qualities, and other commercially important traits. The inability of proteomics to investigate all protein components has been one of the key problems for proteomics applications in crop breeding, which may be due in part to the technology itself, protein extraction inefficiencies, proteome complexities, and biological variations. Protein arrays provide an alternative explanation and have the potential to find biochemical pathways and novel protein markers in high-throughput applications. Because of the use of varied plant genotypes and sample processing, as well as the different proteomic methodologies, it is currently difficult to draw consistent conclusions from different crop proteomics investigations. Finally, the knowledge gained from proteomic research will aid in the modification of traditional crop breeding architecture, allowing the creation of agricultural plants with higher attributes (stress resistance, disease tolerance, and high yielding), achieving our goal of increased crop productivity.

## Conflict of interest

There are no conflicts of interest declared by the authors.

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