

Proteomic and Metabolomic Insights into Plant Stress Responses

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Agriculture plays a pivotal role in the economic foundations of several nations. In addition to its function in providing nutrition, nourishment, and critical resources, agriculture also plays a crucial role as a major source of employment for a large portion of the world's population. Based on data provided by the Food and Agriculture Organisation of the United Nations (FAO), it is apparent that a majority exceeding 60 percent of the global population relies on agriculture as a primary source of sustenance. In the majority of poor nations, agriculture plays a pivotal role in driving national revenue, although its significance is somewhat less pronounced in affluent nations.

According to projections, the global population is anticipated to see a 30% increase, ultimately reaching a total of 9.7 billion individuals by the year 2050. In order to achieve global food security, the Food and Agriculture Organisation of the United Nations (FAO) has projected that there is a need for a 70% increase in global food production.

India is primarily identified as an agrarian country, wherein a substantial majority of its rural households, over 70%, largely depend on agriculture and its related industries as their main source of livelihood. In the Indian context, it is evident that a considerable percentage of cultivable land, namely 47.3%, is allocated to a majority of small-scale farmers, who constitute around 87.2% of the overall farming population. With over 126 million farmers and an average landholding size of 0.6 hectares, smallholder farmers control more than 74.4 million hectares of land in total.

Biotic and Abiotic stress

As the population grows, there is a corresponding increase in the need for food. The present and anticipated worldwide need for food necessitates a significant expansion in crop production in regions with less favourable agricultural conditions.

Biotic and abiotic stresses are substantial limitations that hinder the growth, development, and productivity of plants. Food security is presently a significant problem and source of apprehension for agronomists and plant physiologists on a worldwide scale. Biotic stress in plants is caused by living creatures, including viruses, bacteria, fungi, nematodes, insects, arachnids, and weeds. Abiotic stressors, such as drought, salt, heat, radiation, severe weather conditions, floods, and heavy metals, are significant agricultural limitations that impact plant growth, development, and productivity. Biotic stressors continue to pose the most significant limitation on crop productivity. The investigation of abiotic stress is undertaken because of its prominent role in causing worldwide crop loss, resulting in a reduction in average yields for the majority of agricultural plants by over 50%. Additionally, it hinders plants from attaining their full genetic capacity.

Stress Management Strategies of Plants

Plants, being immobile creatures, have evolved effective response techniques to mitigate, endure, or adapt to many forms of stress throughout their evolutionary history. Plants encounter a wide range of stress stimuli, which frequently trigger common cell signalling pathways and cellular responses. These responses include the synthesis of stress proteins, the enhancement of antioxidant mechanisms, and the build-up of suitable solutes. Stress can be conceptualised as an external stimulus or influence that falls outside the typical boundaries of homeostatic regulation within a specific organism. When the capacity to tolerate stress is surpassed, various mechanisms are triggered at the molecular, biochemical, physiological, and morphological levels. Once the stress is effectively managed, a new physiological state is established, leading to the restoration of homeostasis. When the stress is alleviated, the plant has the potential to revert back to

its original state or adapt to a new physiological condition.

The advent of modern technology has given rise to a range of multidimensional omics disciplines, including proteomics, metabolomics, and others. These disciplines focus on the molecular constituents of cellular life. The progress made in genomic research has facilitated the identification of many gene families and processes that influence the ability to withstand abiotic stresses, ultimately leading to increased crop productivity. The field of systems biology, a very nascent area within the life sciences, is addressing the challenge of integrating many omics approaches to yield substantial biological insights. The integration of systems biology with virtual experiments offers a means to visually represent and understand the mechanisms by which plants adapt to abiotic stress. Furthermore, the development of climate-resilient cultivars for consistent and productive yields in challenging climatic conditions requires the incorporation of diverse areas of expertise from several disciplines.

Proteomics and Metabolomics approaches

Proteomics and metabolomics technologies are employed to investigate alterations in proteins and metabolites in response to stressful conditions. Over the course of the past decade, much study has been conducted to get a comprehensive understanding of the underlying mechanisms by which plants respond to various forms of environmental stress. The current body of research on abiotic challenges is limited due to insufficient investigation into the regulatory mechanisms that activate various genes and pathways in response to abiotic stimuli. The complexity of plant stress response and adaptation processes is evident based on the participation of these genes.

Proteomics

As previously stated, the word "proteome" encompasses the whole set of proteins that are expressed by the genome, including post-translational modifications. The characteristic being discussed exhibits neither constancy nor homogeneity. The

primary emphasis of proteomic investigations frequently involves protein identification, quantification, localization, and post-translational modifications (PTMs), as well as the examination of functional, structural, and protein-protein interactions. Proteomics not only provides insights into the complexity of biological systems, but it also has significance in understanding cellular viability and their adaptive responses to diverse environmental conditions. Although the investigation of post-translational modifications (PTMs) has predominantly focused on transcript-level analysis, it is worth noting that assessing protein abundance, which is intricately linked to enzymatic activity, might potentially provide a more dependable signal of protein function.

The measurement of plant stress tolerance was often conducted by assessing the whole proteome. However, later research has expanded the scope of proteome-related investigations to include other aspects such as the organellar proteome, phosphorproteome, nuclear proteome, cell wall proteome, and proteo-genome. Therefore, proteome profiling is conducted using different methodologies, including mass spectrometry (MS), which involves the analysis of protein fragments to determine their charge and mass and identify any modifications in the peptide sequence. Other techniques, such as matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) and two-dimensional gel electrophoresis (2-DGE), are also utilised. Recent advancements in proteomics will contribute to the discovery of more regulatory target proteins, facilitating the development of stress-tolerant crops that exhibit enhanced yield and quality.

Proteomics Applications in Plant Stress Responses

Yousuf et al., 2016 analyzed salt stress-induced modulations in the shoot proteome of salt-sensitive (*Pusa Varuna*) and salt-tolerant (CS-52) genotypes of Indian mustard and total of 320 spots were identified, out of which, only 21 proteins showed more than two-fold change in abundance. Out of these 21, 13 were found to be with increased intensity while 8 were downregulated.

Jia *et al.*, 2015 conducted comparative proteomic analysis of seedling leaves of *Brassica napus* exposed to 200 mM NaCl for 24 h, 48 h and 72 h and found 42 proteins, involving few novel salt stress responsive proteins like Cinnamyl alcohol dehydrogenase (CAD) and N-glyceraldehyde-2-phosphotransferase were showing differential expression.

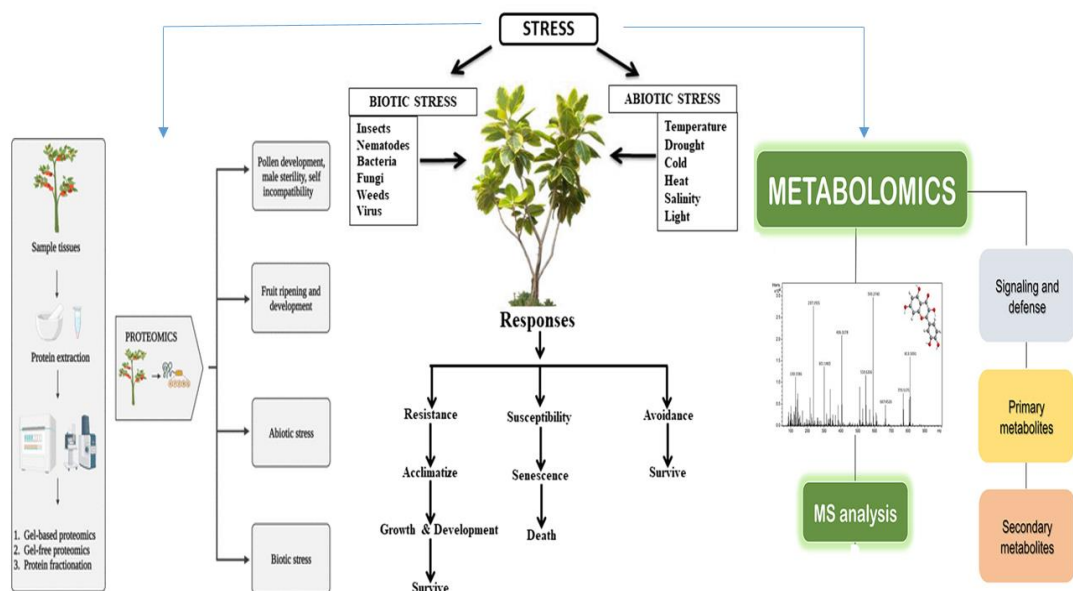
Metabolomics

Metabolomics facilitates the evaluation of biological processes within intricate environmental conditions. The estimated number of metabolites in the plant kingdom, encompassing a wide range of polar to nonpolar, volatile to non-volatile compounds, surpasses 200,000. In order to comprehensively examine the chemical characteristics of a metabolite, it is essential to combine many analytical techniques, given the vast range of its variability.

The utilisation of separation-based techniques, such as liquid/gas chromatography (LC/GC) in combination with mass spectrometry (MS), nuclear magnetic resonance (NMR), and Fourier transform ion resonance (FTIR), has significantly contributed to the analysis and measurement of metabolites in both untargeted and targeted approaches. Targeted metabolomics involves the deliberate selection of a limited number of chemicals, accompanied by reference standards, to ensure accurate analysis and clear identification of metabolites. On the other hand, untargeted metabolomics seeks to characterise a wide array of metabolites that possess distinctive characteristics.

Metabolomics Applications in Plant Stress Responses

The term "plant stress" refers to any modification in growth conditions that disrupts the metabolic balance and requires the adjustment of metabolic pathways through acclimation. Metabolites exhibit spatial distribution within many tissues, organs, and cellular compartments. Plants are subject to several environmental conditions, including both abiotic and biotic stressors, which in turn lead to changes in their metabolite composition. These factors impede the general growth and development of plants and reduce productivity, posing a threat to food security, particularly in light of the challenges posed by the global population expansion. Metabolomics has the potential to provide valuable insights into plant metabolism throughout the developmental process and in response to diverse stressors. This is achieved by identifying a range of substances, including stress metabolism derivatives, stress signal transduction molecules, and molecules associated with the acclimation response of plants. Furthermore,



metabolomics can contribute to a more comprehensive understanding of stress biology in plants by facilitating a guided explanation of these processes.

Primary metabolites play a crucial role in the growth and development of plants and exhibit a high

degree of conservation in their molecular structures and abundances across various plant species. In contrast, secondary metabolites and their regulation are more susceptible to environmental fluctuations, such as variations in light intensity, temperature, water availability, salinity levels, exposure to ultraviolet radiation, nutrient deficiencies, the presence of heavy metals, oxidative stress, and interactions with neighbouring plant species.

In a ground-breaking study, *Rodrigues Neto et al. 2023* did a multi-omic integration analysis on Purslane plants (*Portulaca oleracea* L.) that had been exposed to salt stress mitigation. This analysis is of utmost importance owing to the global adaptability of Purslane, which is attributed to its therapeutic benefits.

The study conducted by *Wu et al. 2022* aimed to investigate the effects of shade on blueberries as a means to mitigate the adverse effects of excessive heat stress that occur during the peak production period. The application of moderate shade at a level of 50% was seen to have a positive impact on plant development as well as the enrichment of photosynthetic pathways and the promotion of flavonoid production. This finding suggests that employing this technique might be an efficient method for enhancing blueberry farming under high-temperature conditions.

Conclusion

Various abiotic stressors have been observed to significantly impact the development and production of crops. Various omics-based methodologies, including proteomics, metabolomics, and others, have proven effective in investigating the plant's reactions to stress, either in isolation or in conjunction with one another. Upon the onset of stressful conditions, plants

undergo physiological adaptations through modulating metabolic pathways and genetic regulatory mechanisms, eventually resulting in the activation of novel genetic expressions. Therefore, it is important to clarify the genetic composition and its corresponding role in order to unveil the plant's reaction in the face of stress. The feasibility of this outcome is contingent upon advancements in biotechnology and the use of omics tools and procedures.

References

- Rodrigues Neto, J.C., Salgado, F.F., Braga, Í.D.O., Carvalho da Silva, T.L., Belo Silva, V.N., Leão, A.P., Ribeiro, J.A.D.A., Abdelnur, P.V., Valadares, L.F., de Sousa, C.A.F. and Souza Júnior, M.T., 2023. Osmoprotectants play a major role in the *Portulaca oleracea* resistance to high levels of salinity stress—insights from a metabolomics and proteomics integrated approach. *Frontiers in Plant Science*, 14, p.1187803.
- Wu, Y., Huang, Z., Zhang, C., Shi, C., Lyu, L., Li, W. and Wu, W., 2022. Comparative analysis of the morphological, physiological, proteomic, and metabolic mechanisms of the “Biloxi” blueberry response to shade stress. *Frontiers in Plant Science*, 13, p.877789.
- Yousuf, P. Y., Ahmad, A., Ganie, A. H., & Iqbal, M. (2016). Salt stress-induced modulations in the shoot proteome of *Brassica juncea* genotypes. *Environmental Science and Pollution Research*, 23, 2391-2401.
- Jia, H., Shao, M., He, Y., Guan, R., Chu, P., & Jiang, H. (2015). Proteome dynamics and physiological responses to short-term salt stress in *Brassica napus* leaves. *PLoS One*, 10(12), e0144808.

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