



# OMICS APPROACH FOR STRESS TOLERANCE IN CROP PLANTS: A REVIEW

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## Abstract

As a result of increased incidence of global climatic changes many crop plants have become more vulnerable to various abiotic and biotic stresses or different combinations or interactions of stresses. Before pre-genomic era, conventional plant breeding approaches were the most prominent ones to improve the genotypes with specific traits such as yield, disease resistance and nutritional value etc. With the advent of various *omics* tools such as genomics, transcriptomics, phenomics, proteomics and metabolomics etc., the traditional breeding approaches have been greatly revolutionized in the late 20<sup>th</sup> and 21<sup>st</sup> century. The advances in omics approaches and next generation genetic engineering technologies such as plastid engineering and miRNA technology have great potential to understand the plant responses against different biotic and abiotic factors in order to improve the stress management/tolerance in crops. In this context, the integrated uses of the all the omics approaches could be useful in delineating the complex mechanism of stress tolerance in various crops in future.

**Key words:** Genomics, Transcriptomics, Proteomics and Metabolomics.

## Introduction

The productivity of all the agricultural crops gets somewhat limited due to many abiotic and biotic stresses across the world. At the same time, the human population is growing continuously at a fast pace and United Nations reports depict the current world population as 7.6 billion and it is predicted that the population will be increased by 9.8 billion in 2050 and 11.2 billion in 2100 (UND, 2017). Hence, sooner or later, there will be a great challenge of proportionate demand of food to feed the global population. The yield of crops has decreased by about 25% at global level as a result of biotic stress (Savary *et al.*, 2012). Abiotic stresses are responsible for yield losses of almost more than 50% in major crops (Acquaah, 2007). Climate extremes *i.e.* drought, flood, heat waves, cold streams and cyclones etc. have been major abiotic stresses in crop plants. Inter-governmental Panel of Climatic Change (IPCC) reports describe that the abiotic stresses will increase in the future as a result of global climate change (<https://www.ipcc.ch>). Various defense responsive genes, transcription factors and proteins have been identified that are found in activated state in the plants during biotic and abiotic stresses. In recent years, various technologies from conventional to omics approaches *viz.* Genomics, transcriptomics, proteomics, metabolomics, bioinformatics etc. are being used to improve the crop plants. These tools facilitated the understanding of mechanism of biotic and abiotic stress responses and helped in identification of defense related genes, proteins and responsive molecules that consequently played a major role in management of stress tolerance in various commodity crops such as sugarcane, rice,

wheat, barley, sorghum and vegetable crops etc. Further epigenetic control at the DNA and histone levels, and gene regulation by non-coding RNA (miRNA) also appear to be vital in state of stress.

## Stresses that affect the plants

Plant stress can be defined as unfavourable constraints imposed by either biotic molecules or abiotic factors (environmental factor) which affect the homeostasis of plant cells and eventually limit the growth of plants as well as result in loss of agricultural productivity. Plants' nature to avoid or tolerate the stresses has brought many changes at morphological, molecular, biochemical and physiological level as a continuing process of evolution. On the basis of number of interacting factors, stresses can be divided into three classes such as single, multiple individual, and combined stresses. A single stress is characterized by only one stress factor and has an effect on plant growth and development, whereas multiple stresses are having effects two or more stress factors taking place at different time periods without any overlap (multiple individual). The last one *i.e.* combined stresses are attributed by two or more stresses factor taking place simultaneously with at least some degree of overlap between them (combined). The co-occurrence of bacterial and drought stresses is an example of combined biotic and abiotic stress; drought and heat stresses in hot climatic condition is an example of a combined abiotic stress, whereas a bacterial and fungal pathogen attacking a plant simultaneously represents the example of combined biotic stress (Pandey *et al.*, 2017a).

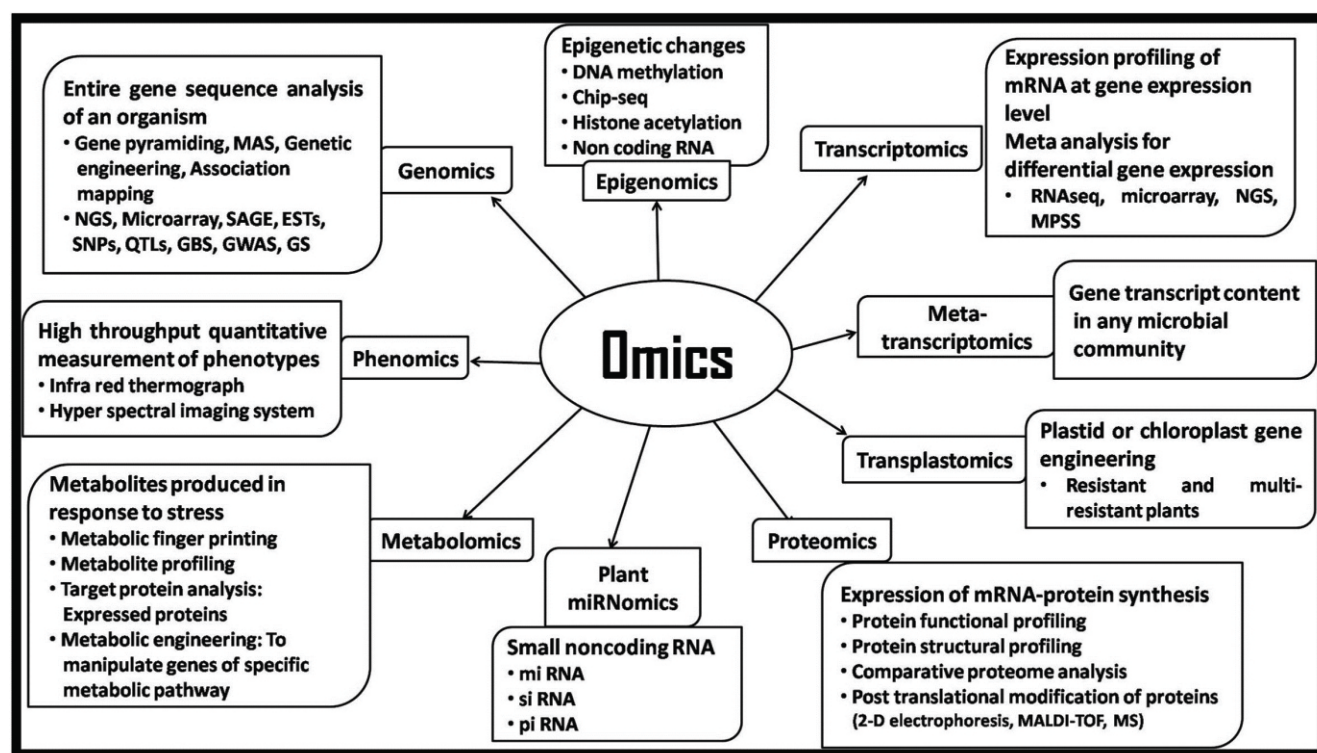
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The signalling cascades that trigger ion channels, kinase cascades, and production of reactive oxygen species (ROS), accumulation of phytohormones *i.e.* salicylic acid, ethylene, jasmonic acid, and abscisic acid inside the plant cell integrity are generally initiated due to biotic or abiotic stress conditions. These signals eventually induce functional groups of genes that act either by protective function or regulatory and assist the expression of specific defense genes and proteins and ion molecules that lead to the generation of the overall defence reaction (Jaspers *et al.*, 2010).

## Omics approaches to mitigate stress response

Omics approaches have diverse range of applications and offer great biological insights. Currently a number of *omics* branches have been introduced in biology such as genomics, phenomics, transcriptomics, proteomics and metabolomics to name a few that have wide applications in plant science. *Omics* approaches are playing significant role in understanding of numerous features of plant stresses imposed

by abiota and biota in nature. The omics technology has assisted in identification of trait linked genes that provide more logical conviction, leading to improved varieties and dissecting the mechanisms of fungal, bacterial, viral, insect and weed resistance. In addition, facilitates the information of molecular mechanism of different abiotic stresses in plant (drought, water, salt stress etc.). Thus, these are helpful in development of plants which will be resistant to stresses. *Omics* even also facilitated the emergence of systems biology approach for understanding the complex interactions among genes, proteins and metabolites inside the cell and helped in delineating the complex molecular machinery governing growth, cellular development, metabolism and stress responses. This integrated approach is strictly based on chemical analytical methods, computational and bioinformatics analysis and many disciplines of biology, leading to protection and improvements of crop (Perez-Clemente *et al.*, 2013; VonEmon, 2016; Ibraheem *et al.*, 2018).



**Figure 1: Key branches of Omics and their major applications**

The deeper insight into diverse omics approaches (Figure 1) and their important applications with a focus on plant stress response have been delineated as follows:

### Genomics

Genomics is the science which deals with the entire gene sequences of an organism and has progressed enormously over the past decade. It is playing a crucial role towards providing necessary knowledge for stress improvement in crops. Applying the genomics tool, a number of stress related genes have been identified and characterized which are involved in stress response and signalling pathways. The

applied tools of omics, such as gene pyramiding, genetic engineering and marker assisted breeding have tremendously enhanced the ability to generate stress tolerant crops. In plants, expressed sequence tags (ESTs), microarray and serial analysis of gene expression (SAGE) tools are used to search for functional genes. (Perez-Clemente *et al.*, 2103). Single Nucleotide Polymorphism (SNP) has been used for detection of selective traits. The Next generation sequence (NGS) technologies with Illumina or Solexa, Ion torrent personal genome machine (PGM) and Pacific biosciences sequencing methods have also reformed the genomic research through application of an exact and fast phenotyping. Quantitative trait

loci (QTL) mapping has become fast and accurate and can possibly discover rare alleles and give good estimates of the allelic effects in a given genetic background. Bi-parental QTL mapping or association mapping techniques to know the inheritance and genetic variance of complex quantitative traits are also at present (Bekele *et al.*, 2014). However, these are generally more costly and time consuming than getting SNPs data for thousands of markers (D'Hont *et al.*, 2013; Heather *et al.*, 2016).

In *Brassica napus* L., through the genome wide analysis, Wan *et al.* (2017) have identified 38 possible candidate genes in genomic regions related with salt tolerance. These genes comprise of transcription factors, aquaporins, transporters, and enzymes. In *Sorghum bicolor* by genome wide analysis, Chopra *et al.* (2017) identified a potential gene network responsible for adaptation to cold and heat stresses at the stages of plant development. Genomics therefore, provides knowledge-based approaches for crop plant biotechnology by enabling precise and controllable methods for molecular breeding and marker-assisted selection, thus promoting the development of new resistant crop cultivars.

## Transcriptomics

The complete set of RNA is defined as transcriptome. Transcriptomics is a study of expression profiling of any gene at mRNA level. In other words, it reveals that how the gene expression pattern changes due to internal and external factors *i.e.* biotic and abiotic stresses in crops (Atkinson *et al.*, 2014). Sequencing based approaches (RNAseq), NGS and Microarrays technique are used for transcriptome dissection (Valdes *et al.*, 2013; Johnson *et al.*, 2014). The Massive Parallel Signature Sequencing (MPSS) technique was used to quantify mRNA transcripts for gene expression analysis, however, this technology was short-lived and not widely accepted in plants due to cost limitations.

An analysis of changes at the transcript level can be used to identify new signaling proteins and metabolic processes which are important for plant development and also for stress tolerance. Powell *et al.* (2016) used an RNAseq based transcriptomics study in bread wheat (*Triticum aestivum* L.) to monitor expression patterns during infection of a pathogenic fungus *Fusarium pseudograminearum*, that causes crown rot disease in cereals. Rasmussen *et al.* (2013) studied the transcriptome variations occurring in 10 ecotypes of *Arabidopsis* under different stresses and double combinations of abiotic stresses. This study reveals that *Arabidopsis* plants are able to cope up the combination of stresses too. Transcriptome analysis is applied for the expression of thousands of genes and their co-expression partners under various stresses in different crops (Sharma *et al.*, 2013; Rest *et al.*, 2016). Meta-analysis for transcriptome data can be used to assess differential gene expression between normal and stress conditions and to identify such genes whose expression products act as key molecules in response to a particular stress (Tseng *et al.*, 2012; Shaar-Moshe *et al.*, 2015). Thus, the plant genomics and transcriptomics studies provide drafts of complete genes and their transcribed mRNA under specific stress states.

## Proteomics

Proteins are translational products of cell that are responsible for many cell functions. Proteomics approach is used to determine the mRNA expression in terms of protein synthesis that governs the gene function. Through protein expression profiling, specific proteins can be identified at a specific time as a result of response to perception of biotic stresses such as fungal, bacterial, viral and insect invasion or abiotic stress such as temperature and drought. This explains the function of expressed proteins in cells. Comparative proteomic studies can analyse the molecular mechanisms for susceptibility or resistance in plants for disease stress, therefore these studies can significantly elucidate the possible relationships between protein accumulation and plant stress acclimation. The post-translational modifications of these proteins assist in knowing the biological function during the growth and development of a plant and or in response to various stresses. Number of transcription factors of several families, associated to abiotic and biotic stress in plants have been identified such as NAC, WRKY proteins, bZIP (basic leucine zipper), MYB/ MYC (Perez-Clemente *et al.*, 2013), ethylene responsive *i.e.* APETALA2/ERFs etc. (Onate-Sanchez *et al.*, 2002; Cao *et al.*, 2018).

Techniques used in proteomics studies include, 2D Protein Electrophoresis, Mass spectroscopy (MS) and matrix assisted laser detection of desorption/ ionization- time of flight (MALDI-TOF) (Rabilloud *et al.*, 2011; Mann *et al.*, 2001). In various crops, proteomics-based studies were performed for identification of changes in the levels of stress response proteins (Li *et al.*, 2017; Zandalinas *et al.*, 2017). Proteome analysis of the watermelon plant roots which have the infection of *Fusarium oxysporum* (responsible for wilting) resulted in differential expression of proteins. Zhang *et al.* (2017) found 690 protein spots, of which 32 proteins were involved in metabolism, stress and showed considerable changes in their expression pattern for defense. In *Jojoba* plant Al-Obaidi *et al.* (2017) identified 45 proteins through the use of gel based proteomic analysis. These proteins were engaged with processes such as photosynthesis, energy and metabolism and also acted in response to biotic and abiotic stresses. Proteomics and functional genomics have played an important role in understanding of various processes including stress tolerance mechanism.

## Metabolomics and Metabolic engineering

Metabolomics is a fast developing tool, that gives complete insight about secondary metabolites, hormones, and signaling molecules produced in plant. Plants when constrained to stresses conditions, make an effort to tolerate the stress state through inducing various metabolic changes (Obata *et al.*, 2012). Metabolomics has been applied to study the metabolites produced in plants under such stresses. Metabolomics currently applied in plant research comprises of metabolic fingerprinting, metabolite profiling and target analysis. Proteomics identifies the gene products produced, while metabolomics determines that the expressed proteins are metabolically active and identifies their biochemical processes and the roles of the various resulting metabolites.



These studies have also been employed to evaluate stress responses in crops such as barley (Widodo *et al.*, 2009), *Citrus* (Djoukeng *et al.*, 2008), *Medicago truncatula* (Broeckling *et al.*, 2005) and *Arabidopsis thaliana* (Fukushima *et al.*, 2011). The metabolome decides the downstream result of gene expression and it is closer to phenotype than transcript expression or proteins. The simultaneous monitoring of metabolic networks facilitates the association of changes resulting from biotic or abiotic stress which can assist in the development of improved cultivars as well as understanding of systems biology (Aliferis *et al.*, 2011; Dixon *et al.*, 2006).

Further, the engineering of metabolites is also emerging as potential approach to improve stress tolerance in plants. Metabolic engineering involves the manipulation of a single gene or genes involved in a specific metabolic pathway. Studies have demonstrated that the manipulation of a single gene involved in a specific metabolic pathway may not be very effective. Therefore, it would be more advantageous to engineer multiple enzymatic reactions to get enhanced tolerance against stress (Reguera *et al.*, 2012).

## Transplastomics

Plastid genome or plastome is double standard circular DNA and its length ranges between 70 kb to 120 kb. It is maternally inherited and prokaryotic in origin. Transplastomics deals with plastid or chloroplast gene engineering studies. Plastid engineering, a next generation gene engineering technology offers desired transgene stacking and high expression of foreign proteins, and is potentially able to generate resistant and multi resistant plants called transplastomic plants. Multi-resistant plants have resistance to plant pathogen, insects and abiotic stresses. Chen *et al.* (2014) developed the *Nicotiana benthamiana* transplastomic plant which was resistant to insects, pathogen and abiotic stress. A study from tobacco transplastomic plant suggests the over expression of a theta class AtGSTT (*Arabidopsis thaliana* glutathione transferase) resulted enhanced tolerance to abiotic stress (Stavridou *et al.*, 2019).

## Phenomics

Plant phenomics deals with reliable, automatic, sophisticated and high throughput technology for quantitative measurement of various phenotypes to create high dimensional data of living being. The phenotypic high throughput technologies are very useful in biotic and abiotic stress management. Infrared thermography (IRT) applied to screen the number of wheat genotypes in response to salt stress (James and Sirault, 2012). The high throughput technology such as hyperspectral imaging system was used to estimate the progression of fire blight infection in young apple (Jarolmasjed *et al.*, 2019). Wedeking *et al.* (2017) applied the IRT to observe the leaf temperature and transpiration in *Beta vulgaris* plants under progressive drought stress.

## Metatranscriptomics

Metatranscriptomics is defined as total gene transcript content (mRNA) in microbial community. It varies according to time and environmental changes. Metatranscriptomics

deals with techniques employed to get the total expression profile in a community and to know the information about the dynamics of gene expression pattern during environmental states. It provides information about adaptive mechanism in complex communities as well as genetic changes that occur during environmental changes. The comparative metatranscriptomics study by Hayden *et al.* (2018) of wheat rhizospheric microbiome was performed in disease suppressive and non-suppressive soils for fungus *Rhizoctonia solani* AG8. The study suggested different expression patterns of wheat rhizospheric microflora in suppressive and non suppressive soils and a molecular play among plant-microflora-pathogen interaction which promoted rhizospheric microflora to decrease the *Rhizoctonia solani* AG8 infection in wheat crop.

## Epigenomics

Epigenomics deals with epigenetic changes that regulates gene in genome. DNA methylation, histone acetylation and non coding RNA that contribute in structure remodelling of chromatin eventually up regulate and down regulate the gene. A study performed with MSAP (methylation sensitive amplified polymorphism) analysis in salt tolerant and salt sensitive cultivars of foxtail millet under saline conditions identified various polymorphic MASP fragments. The study described DNA methylation during regulation of gene expression under salinity (Pandey *et al.*, 2017b).

## Plant miRNomics

Micro RNA in plant are small non coding RNA, consisting of 21 to 24 nucleotides. Various miRNAs identified in plants, have played critical roles in developmental processes and tolerance to various biotic and abiotic stresses (Xin *et al.*, 2010). The miRNA plays significant regulatory functions in development and stress response in different crops by negatively influencing post-transcriptional gene expression. In potato plant, drought responsive miRNA named as miR171, miR171b and miR171c (miR171 family) have been identified by Hwang *et al.* (2011).

## Conclusion

Various omics approaches have emerged as potential tools to understand the plant responses against different pathogens and abiotic factors. In addition, Omics approaches have also been helpful in stress tolerance management. The desired phenotype of any crop plant is the ultimate aim of plant breeding; therefore, intense phenomic analysis *vis-à-vis* diverse omic tools will give successful management of stress resilience in plants. The integrated uses of the multi-omic approaches could be useful in understanding the mechanism of stress tolerance in various crops in future.

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