



Metabolomics, a Potential Way to Improve Abiotic Stresses Tolerance in Cereal Crops

Muhammad Usama Noman and Salman Azhar

Department of Plant Breeding and Genetics, University of Agriculture Faisalabad Pakistan

*Corresponding author: musamanoman@gmail.com

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ABSTRACT

Rapid Climate change is causing the highly diversified effect on both quantity and quality in extensive area crops. All abiotic stresses are based on ecological conditions due to any small change that can create a future challenge for us. Metabolomics is a very valuable approach and powerful tool that helps to understand plant reaction stimuli. A range of variable analytical approaches (GC-MS, LC-MS, CE-MS, NMR) has been utilized to inspect the action of the metabolites. It promotes recognizing and understand the markers of bio-stimulants action. Plants have developed complex systems to recognize external signals to adjust according to fluctuating environmental factors for their survival in stressful environments. Plants respond to abiotic stresses by producing various metabolites and by expression of certain stress related genes. Comprehensive data recognition and identification of complicated metabolites is a challenge however appropriate software tools application promotes the accuracy with big data-driven precision. The review articulates hurriedly the basic aspects relating to identifying the pathways through valuable tools in metabolomics.

Key words: Biotic Stress, Abiotic Stress, Metabolomic Assisted Breeding, Metabolic Pathways, Nuclear Magnetic Resonance Spectroscopy, Metabolites, Reactive Oxygen Species.

INTRODUCTION

Metabolomics is a new and exciting approach in omics technologies that has recently gained traction. Crop enhancement techniques have been widely used. Abiotic stress research requires the use of metabolomics. Tolerance, pathogen resistance, robust ecotypes, and metabolomic assisted crop breeding are all examples of metabolomic assisted breeding. Until now, the development of modern metabolomics tools for crop improvement has made tremendous progress (Shulaev et al., 2008; Razzaq et al., 2019).

The identification and quantification of all low molecular weight metabolites in each organism, at a given developmental stage, and in each organ, tissue, or cell type has been identified as metabolomics (Fiehn, 2001; Arbona et al., 2014). The fields of metabolomics, genomics, transcriptomics, epigenomics, proteomics and phenomics have all seen significant advancements in recent years. These 'Omics' methods have improved the precision and efficiency of ongoing breeding programs in developing climate-smart and nutrition-rich germplasm, which is necessary to assure food security (Parry and Hawkesford, 2012). Metabolomics has emerged as a valuable technology for systematic profiling and comparison of metabolites in biological systems, with a

wide range of applications documented in plant sciences (De Vos et al., 2007). The plant kingdom comprises approximately 200,000 different metabolites, the vast majority of which are still unknown. Around 10000 secondary metabolites have been discovered in various plant species, according to estimates. The newly identified metabolites vary structurally. In plant biology, their biochemical properties and functions are extremely significant (Foito and Stewart, 2018). Modern metabolomics platforms are being used to visualize complex biological pathways and uncover secret regulatory networks that regulate crop production and growth. Within biological systems, a metabolome is a complete collection of low molecular weight metabolites. The primary goal of metabolomics research is to identify and quantify small molecules (less than 1500 Da), as well as their chemical structure and association within an organism (Deborde et al., 2017). Plants metabolize a wide range of primary and secondary metabolites with a variety of chemical structures. Plants need primary metabolites for growth and development, but secondary metabolites serve a different function, and both types of metabolites are involved in plant stress response mechanisms (Hong et al., 2016). Understanding chemical signals as plants grow and evolve requires metabolomics (Sharma et al., 2018).

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One of the modern world's key concerns is to deal with the sustainable production of food to feed an ever-increasing population. According to the Food and Agriculture Organization (FAO), by 2050, crop productivity must have doubled. The productivity forecast of existing crops, however, is not encouraging due to climate and environmental stress conditions (Zafar *et al.*, 2021; Zafar *et al.*, 2022). Several crops' productivity is estimated to collapse by roughly 50% by 2080 in many parts of the world (Zhang *et al.*, 2019). Climate change threatens global agriculture because changes in rainfall, heat waves, and global CO₂ levels cause a variety of abiotic stresses that have a negative effect on food production (Raza *et al.*, 2020). Cereal crops such as rice, maize, wheat, barley, sorghum, oat, and millet account for most of the world's food supply (Balmer *et al.*, 2013). Since both biotic and abiotic stress factors pose a threat to crop production sustainability, abiotic stresses have a greater influence on agricultural productivity of crops such as wheat, soybean, and maize than biotic ones. Indeed, typical climate-related elements like water, temperature, and soil salinity reduce crop output significantly over the world (Ciura and Kurk, 2018). Changes in various cellular metabolic processes, including as glucose, amino acid, and peptide metabolism, are directly linked to abiotic stressors. However, if the metabolic balance is upset, growth, development, and, as a result, yield may be harmed (Das *et al.*, 2017).

As a response, the development of sustainable agriculture techniques, along with more productive and stress-tolerant crop varieties carrying genetic features linked with environmental change adaptation (or high tolerance to abiotic stresses), will be required to sustainably grow high-yielding crops under more demanding environmental conditions, either alone or in combination. (Furlan *et al.*, 2017; Zafar *et al.*, 2020). Intercropping can improve resilience to climate change through increased plant resource utilization i.e. space, nutrition, water, weeds, disease, and insect pest reduction (Manan *et al.*, 2022).

Importance of Metabolomics in Plant Biology

Metabolomics is one of the emerging and fascinating approaches of omics tools that is now widely used for crop improvement. Metabolism is important for the study of abiotic stress tolerance, resistance to pathogens, strong ecotypes, and preparation of metabolic assisted breeding of various types of crops (Shulaev *et al.*, 2008).

To date, there have been significant advances in the development of modern metabolomic tools to study the metabolomic pathways of crops. There are 200,000 compounds, most of which are still unknown. An estimated 10,000 secondary metabolites have been discovered in various plant species (Foito and Stewart, 2018). The discovered metabolites are structurally different in biochemical properties and function and are considered very important in plant biology. Metabolome is a combination of with low molecular weight metabolites in biological systems. Metabolic research is primarily concerned with the identification and reduction of molecules as tiny, structural chemical molecules and their functions within the organism (Deborde *et al.*, 2017).

Many metabolites are unique and play an important role in controlling crop performance and nutritional quality. Plant growth in different environmental conditions

is affected by the synthesis of many metabolites. Ecological metabolism involves the study of the interaction of plants with their environment. This is done based on a careful estimate of the levels of metabolites under a specific plant environment to determine their effects on plant adaptation and any changes in their genetic makeup. It provides a platform for examining interactions between the environment and biology to measure plant performance in detail. Another important metabolic application, called ecological metabolism, is determined by analyzing the biochemical interactions of plants in transient and endemic systems. This biochemical nature explains many important environmental phenomena, such as the effects of parasitic loads, the disease, and its infection. It also helps to assess the various effects of non-living factors on the interaction between two traffic levels or the relationship between specific ones. Changes in the concentration of multiple metabolites may have mechanical implications for biochemical networks that explain the phenotypic and physiological feedback of plants to environmental fluctuations. The full potential of ecological transformation has not yet been explored. Biological and non-biological pressures adversely affect crop production and lead to a large reduction in global annual crop production. Metabolic tools can be integrated with mode tools such as genomics, transcriptomics, and proteomics to combat inanimate biotic stress in plants (Liu *et al.*, 2019).

Metabolomics help in analyzing the various metabolites of exotic and endogenous plants under extreme climatic pressure and is a key in understanding plant biology. The application of Omic-based strategies help us to understand the process of pressure regulation in plants. Plant metabolism consists of two types of metabolites: primary and secondary metabolites. The metabolic profile of primary and secondary metabolites provides extensive knowledge of the biochemical processes found during plant metabolism. Some primary and secondary metabolisms of plants can interact with very complex metabolic pathways. Successful identification, diagnosis, and diagnosis of these metabolites is possible through advanced metabolic instruments such as gas chromatography mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and nondestructive nuclear magnetism. Resonance spectroscopy (NMR) Primary metabolites are important for the biosynthesis of lipids, sugars, and amino acids in plants. They mediate the carboxylic acid cycle and the glycolysis cycle during photosynthesis, thus affecting plant growth and development. Changes in the synthesis of primary metabolites can lead to inactivation of photosynthetic function and failure to regulate asthmatic balance in plants. Primary metabolites leads to the production of secondary metabolites such as flavonoids, atropine, carotenoids and phytic acid. These are not essential for plant survival and are produced in response to various stress conditions such as high temperatures, cold, drought, salinity, and insect breeding. In addition, secondary metabolites include reactive oxygen species (ROS), antioxidants, and common enzymes (Seigler, 1998).

The plant metabolite consists of certain secondary metabolites such as phenolics (10,000 10,000), alkaloids (21,000), and terpenoids (> 25,000) that provide biotic / abiotic stress tolerance. Recently, many of these

compounds have been discovered as unique biomarkers that measure plant performance in stressful environments and serve as key components in many crop improvements programs. During plant ontogenesis, primary and secondary metabolites are formed by permanently complex biochemical reactions, so it is important to discover the unique metabolic biochemical processes present in plant organisms. Due to the diverse chemical synthesis, extensive production in cell components, and the complex nature of metabolites, no metabolic tool can be used for complete metabolism profiling. Although a number of methods of metabolome extraction and successful combination of analytical tools for successful metabolic profiling are widely used modern analytical operating systems for decoding the entire metabolism of any plant are still in the early stages (Rodziewicz *et al.*, 2014).

Metabolism has the potential to accelerate the selection of augmentative ingredients and a variety of elite products. Metabolic integration with modern plant genomic tools, such as genotype sequencing (GBS), genome genetic variables and whole genome sequencing, opens interesting horizons for crop improvement. Different cells, which are involved in different cellular processes, are used, thus revealing the whole anatomical structure of this cell.

Abiotic Stresses and Responses of Metabolites in Energy Synthesis Pathway

Abiotic stresses negatively influence the homeostasis & photosynthesis metabolic pathways (Soares *et al.*, 2018; Yadav *et al.*, 2018). Photosynthesis is an energy-giving pathway in plants that directly affects the yield. Abiotic stress badly affects glucose synthesis as a basic source of cell functionality. These stresses cause to close the stomata through CO₂ reduction inside the cell. Also slows down the activity of Ribulose 1-5 bisphosphate carboxylase (RUBISCO) (Kohli *et al.*, 2017). Heavy metals (Cd, Hg, Cu, Pb, Ni, Zn) that are releasing from volcanoes cause soil pollution (Kumar *et al.*, 2020). Heavy metals are capable to change plant's essential elements to biomolecules that cause to reduce the energy supply level (Rascio and Navari-Izoo, 2011; Guo *et al.*, 2016). The shape of chloroplast is highly affected by Cd which reduces the photosynthesis rate (Najeeb *et al.*, 2011). Excess amount of Ni stops the formation of photosynthetic pigments and disrupts its apparatus (Soares *et al.*, 2019). Plants produce SOD, CAT, APX enzymes that promote antioxidant activity (Hasanuzzaman *et al.*, 2020). ROS accumulation can promote the activity of a resistant heavy metal gene by enhancing GHS, PCs, MTs, NA. Global warming promotes the temperature fluctuations worldwide that affect metabolic pathways like photosynthetic, growth pathways (Sharma *et al.*, 2020). There is highly disrupting stress to the photosynthetic system is heat stress (Mathur *et al.*, 2020). Chilling temperature also disturbs the energy synthesis pathway (Hou *et al.*, 2016). Low temperature damages the working of guard cells due to which stomata are closed and internal cell CO₂ concentration adversely affected (Allen and Ort 2001). In biochemical responses, the plant accumulates the MDA, H₂O₂. All of the enzymes and their activity are shown in fig1. Metabolomic studies conformed that transgenic rice ability to uptake more CO₂ and the high rate of photosynthesis process (Sakamoto and Matsuoka, 2008). There is another abiotic stress that is

enhancing through increasing temperature is drought stress. Water is an essential ingredient in the photosynthesis mechanism, but drought causes water and CO₂ loss (Rivas *et al.*, 2016; Chen *et al.*, 2017). Light is considered a key stress factor that harms plant development (Wang *et al.*, 2017). Low light harms the rate of photosynthesis by activating the stomatal conductance and CO₂ concentration within the cell. In response to light plant induce plastoquinone-9.

Plants respond to environmental stresses by the multifaceted signaling pathway of molecules. Every systematic pathway in plants is based on metabolites activity due to which is essential to study for the maintenance and enhancement of quality and quantity. Plants produce several types of antioxidants by the activity of abiotic stresses like ascorbic acid, saponins, flavonoids, glycosylates, phenylpropanoid, and phenol amides (Nakabayashi and Saito, 2015). Flavonoids access and oxidative stress have Spatio - temporal correlation (Hernández *et al.*, 2009). It has been suggested that there is a correlation between metabolite accumulation and gene expression (Tohge and Fernie, 2020). Most of the sugar and water dissolving metabolites are stored in vacuoles act as antioxidants against abiotic stresses (Obata *et al.*, 2012). ROS (Reactive oxygen species) act as a biological marker but during any type of abiotic stress it accumulates. ROS induces hydrogen peroxide, superoxide anion radical, singlet oxygen, hydroxyl radical that cause protein, lipids damage. But sometimes enhances the stress signaling pathway to reduces the damage (Hasanuzzaman *et al.*, 2020). Their activity is based on the enzymatic and non-enzymatic antioxidant system so that play an important Osmo-protectant role during abiotic stresses.

Assessment and Data Analysis Approaches in Metabolomics

In the plant kingdom, about 200,000 types of metabolites exist but still, a huge amount is unrevealed (Foito and Stewart, 2014). There are several biotic and abiotic stresses that directly or indirectly affect the plant metabolites. Metabolomics approaches promote the identification of these affected metabolites with other "omics" tools integration (Piasecka *et al.*, 2019). The observation, recognition, assessment, and data generation of these metabolites can achieve through various metabolomics tools (Che-Othman *et al.*, 2019).

There are numerous metabolites characterization and quantification tools but some most common are GC-MS, LC-MS, CE- MS and NMR. GC-MS a multipurpose analytical platform due to its high efficiency and reproducibility (Mastrangelo *et al.*, 2015). It can only be applicable for low molecular weight & volatile compounds. GC-MS approach analyzed Amino acids, Sugars (mono, di, trisaccharide's) and fatty acids, nucleotides, Ester (Koek *et al.*, 2006). Its implementations in target analysis metabolites and metabolomics (Nassar *et al.*, 2017). LC-MS are more applicable than GC-MS in the Clinical field for separation and analysis. The sample having a higher value than the threshold level re-analyzed by more particular LC-MS. LC-MS essay having the ability to multiplex numerous analytes in a single run with less cost (Pitt, 2009). CE- MS approach analyzed Amino acids, fatty acids, steroids, polar metabolites, and organic acids.

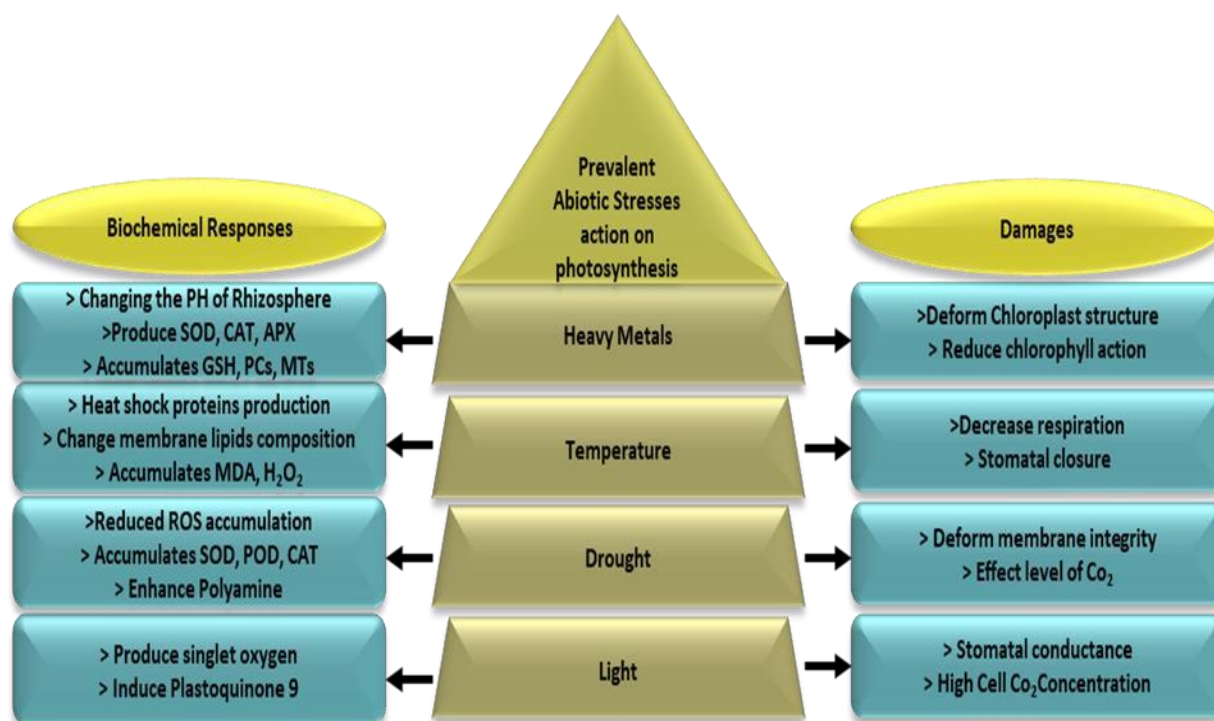


Fig. 1: Bio stimulants for Plant Growth and Mitigation of Abiotic Stresses: A Metabolomics Perspective.

SOD (Superoxide dismutase, an enzyme destroys the destructive molecule of oxygen within the cell).

CAT (Catalase, promote signaling within the cell and normalize cell activity by decomposition of hydrogen-peroxide).

POD (Peroxidase, A haem-containing enzyme that Destroy H_2O_2).

APX (Ascorbate peroxidase, Catalyze L-ascorbate & Hydrogen peroxide).

GSH (Glutathione, support in Fight that against free radicals).

MTs (Mesial temporal sclerosis, Stabilize homeostasis activity within the cell against environmental stresses).

MDA (Malondialdehyde, A aldehyde act as a biomarker to estimate the level of oxidative stress).

H_2O_2 (Hydrogen peroxide, most stable predominantly producing reactive oxygen species ROs).

CE-MS a powerful tool that is used to allocate the charged compounds of a wide analytical range in biological samples (Indenburg *et al.*, 2014). In CE-MS poor concentration sensitivity was removed by porous tip sheathes interface and microbial interface because of metabolic coverage (Lindenburg *et al.*, 2014; Ramautar *et al.*, 2012). Its potential is strongly based on the reliability and sensibility of interfacing procedures like the approach of sheath liquid interface. A valuable step in CE-MS is interfaced versatility as a concentration of BGE changes with the type of interface (Chen *et al.*, 2017). Its implementations in target analysis and profiling of metabolites (Hou *et al.*, 2016). The UPLC-MS gives reservation time, accurate molecular mass, and MS-MS data of fragmentation for structural clarification of biomarkers and other metabolites (Jandrić *et al.*, 2013). NMR is high-throughput, non-destructive, inherently quantitative, automatable, large-scale studies of metabolomics than GC-MS and LCMS. It can be analyzed polar and non-polar metabolites more efficiently than other techniques. NMR supports imaging and analysis in observing metabolites samples by MRS and MRI. At last, NMR is one of the leading technologies than others in metabolomics (Jeong *et al.*, 2017).

Softwares or Tools for Data Analysis

Powerful software approaches are required for a large amount and variety of data generation in metabolomics (Cambiaghi *et al.*, 2016). A lot of tools such as GC-MS, LC-MS, NMR are used in metabolomic studies.

VOCcluster (use for organic compounds identification and density-based non-linear clustering), Wipp (data processing used to distinguish among peaks of real peaks and false peaks on the bases of algorithmic parameters) autoGCMDDataAnal (A software of data processing and data analysis), PyMS (its apply for parallel filtering among by row & by column data analyzing based on MPI) (O'Callaghan *et al.*, 2012) metaMS (its perform pseudospectral Analysis and keep away from alignment stage (Spicer *et al.*, 2017). MSeasy (A metabolite detector performing baseline rectification, smoothing, peak observation and deconvolution) tools are used for data analysis in GC-MS. Peakonly (it is an artificial neural network utilizer to recognize the region of interest) (O'Shea and Misra, 2020), MassCascade-KINME (it's an open-source data processing library) (LC-MS 3), MS-DIAL (an algorithmic base independent data processing), MetAlign (involve in preprocessing activities), IDEOM (an excel template used for identification and statical analysis of metabolites), mzMatch (Processing data based on PeakML design). GlyReSoft (use for glycans data processing) tools are used in LC-MS for data analysis (Maxwell *et al.*, 2012). The currently used approaches in NMR Data analysis are HastaLaVISTA (An R package that supports NMR Data analysis through Interactive Graphical port) rNMR (A preprocessing region-based interest approach) and MetaboLab (A batch processing that helps to detect the data more accurately through statistical analysis) (Ludwig and Günther, 2011). All tools are shown in Fig. 2.

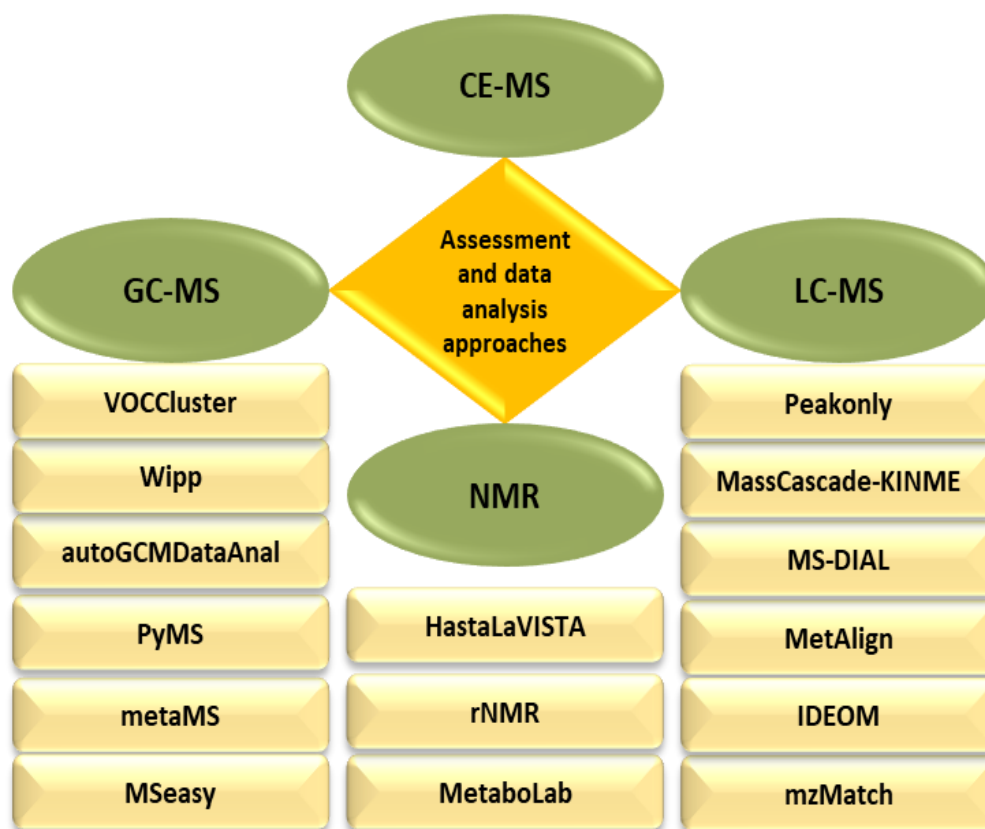


Fig. 2: Basic tools for metabolomics data analysis.

Workflow of Metabolomics

Now a days, abiotic stresses are the main cause of reduced agriculture yield potential in crop plants. Abiotic stresses can be defined as any change in plant growth conditions that affect normal plant growth, development, and physiology. Salinity, chilling, high temperature, drought, waterlogging, and heavy metals are the major abiotic stresses that affect plant growth (Raza *et al.*, 2019). All types of stresses result in significant reduction in yields quantity and quality (Hein *et al.*, 2016). Plants have a very active mechanism for sensing of stress signals and then cellular response are produced to overcome the stress. Both biotic and abiotic stresses produce variations in plant physiological and biochemical processes. When plants undergo abiotic stress, several phytochemicals are produced by the plants to resist against the stress. Different OMICS techniques has been used by scientists to improve the abiotic stresses tolerance in crop plants. Metabolomics is one of these OMIC techniques which is playing its role in improvement of plants. Different plant metabolites variations are under investigation to find out metabolites that can restore the normal plant homeostasis and other metabolic variations which are cause by abiotic stress (Khan *et al.*, 2019). Flow chart showing general steps which are involved in diagnosis and metabolomic assisted breeding for a biotic stress improvement in plants is shown in fig 3.

Metabolomic Assisted Breeding

Drought Stress:

Drought is one of the most important abiotic stresses which adversely affect yield of crops worldwide. Plants

adopt different mechanisms such as abscission of leaves, increased nutrient uptake and reduction in plant leaf area. Similarly closure of stomata helps to reduce the transpiration activity which protect plants from water loss. All these physiological changes help plants to use the water more efficiently which affect the photosynthesis activity as there is reduced CO₂ and stomata closed (Chaves and Oliveira, 2004). Plants produce different polyamines like spermine, spermidine, and putrescine during drought stress (Bitrián *et al.*, 2015). Metabolomic profiling of 6 drought tolerant wheat lines shows that several important metabolites such as -aminobutyric acid (GABA), myo-inositol, threonine, proline, oxalic acid, malic acid, glucose, fructose, and sucrose are produced during drought stress (Marček *et al.*, 2019). High levels of amino acids like lysine, arginine and methionine are also observed when comparative metabolic analysis was done between drought susceptible and drought tolerant wheat lines (Michaletti *et al.*, 2018). These biomarkers can be used to screen drought tolerant lines. Key metabolites produced during different types of stress conditions by different crops are given in table 1.

LC-MS- and GC-MS-based metabolic profiling confirmed the differential accumulation of metabolites in young and matured leaves GC-MS based metabolic fingerprinting of 10 maize hybrids under drought stress revealed low concentration of maltose and erythritol while in rabinose and xylitol no change was observed. Drought tolerant maize lines have high levels of GABA, leucine, glycine, serine, alanine, and tryptophan (Obata and Fernie, 2012).

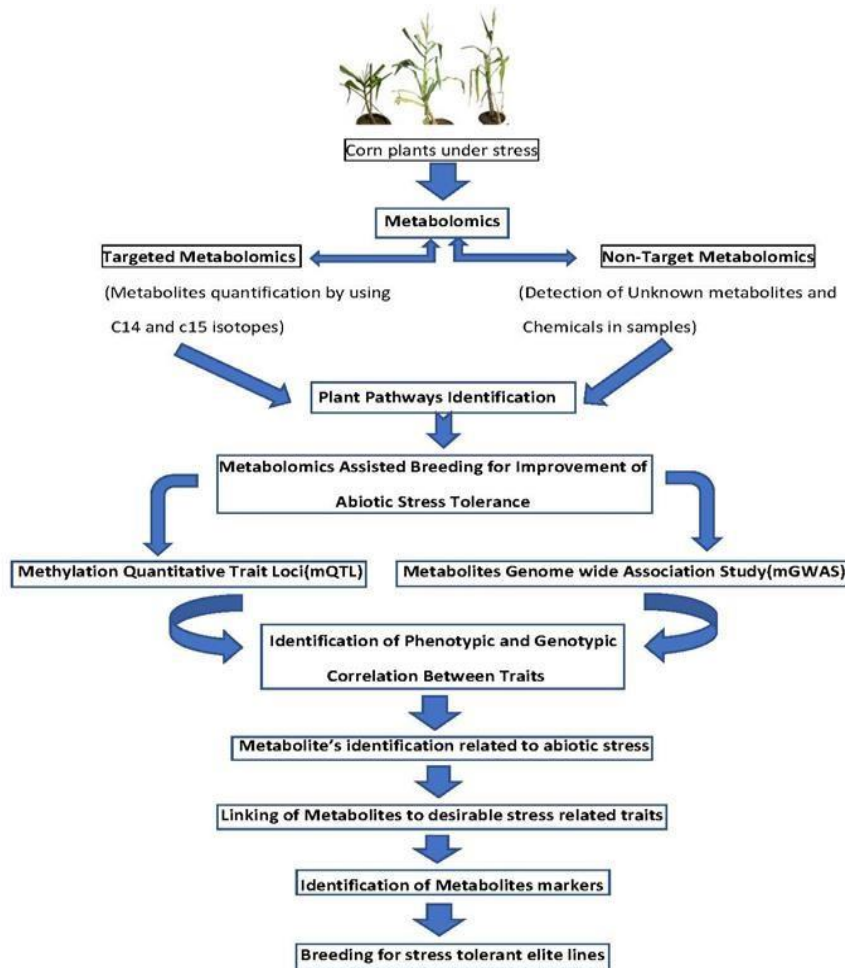


Fig. 3: Work flow diagram of Metabolomics.

Table 1: Major metabolites produced during different types of stress conditions by different crops

Crop name	Stress type	Specific tissue	Major Metabolites produced	Reference
<i>Zea mays</i>	Drought	Immature seeds	Metabolism of lipids, carbohydrates and glutathione cycle	Yang et al., 2018
<i>Triticum aestivum</i>	Drought	Leaf	Glutamine, serine, methionine, lysine and asparagine	Yadav et al., 2019
<i>Glycine max</i>	Drought	Leaf	Glutamine, GABA, allantoin, pinitol and myoinositol	Silvente et al., 2012
<i>Oryza sativa</i>	Salinity	Leaf	Mannitol and sucrose	Chang et al., 2019
<i>Sorghum bicolor</i>	Drought	Leaf	Sugars and sugar alcohols	Ogbaga et al., 2016
<i>Helianthus annuus</i>	Heavy metals	Roots	Fatty acids	Ibarra et al., 2019

Salinity Stress Tolerance

High level of salinity affects nutrient uptake which results in poor growth and development of plants. High concentration of salts cause ion toxicity in plants which makes nutrients and water uptake more difficult for plants (Wu *et al.*, 2013). Different primary and secondary metabolites are produced by the plants under saline conditions. GC-MS base barley roots metabolic profiling indicated high levels of organic acid, proline, sucrose, xylose, and maltose under salinity stress (Gayen *et al.*, 2019). In a recent study, metabolic analysis of rice indicated high levels of sucrose and mannitol, and lower contents of quinate and shikimate in response to salinity stress (Chang *et al.*, 2019). Various metabolomics tools such as GC-MS, LC-MS, and NMR are being used to study metabolic variations in different crop plants like tomatoes, wheat, barley, rice, and maize (Rouphael *et al.*, 2018).

Waterlogging Stress Regulation

Water logging affects crop plants growth and yield. In water logging stress, there is decrease in amount of CO₂ and oxygen which affects the photosynthesis mechanism. Water logging stress for a longer period result in decrease amount of CO₂ by affecting roots of the plants (Barding *et al.*, 2013). In an experiment, rice cultivars resilience was investigated under waterlogging stress. NMR- and GC/MS-based integrated metabolomics tools detected some unique metabolites, including phosphogluconate, phenylalanine, and lactate, that allowed rice plants to tolerant waterlogging stress conditions (Locke *et al.*, 2018).

Heat Stress Regulation

Temperature changes have the potential to do serious harm and disrupt the developmental and plant normal growth process. Under extreme heat, bread wheat

untargeted metabolomics was carried out utilising LC-HRMS. This profiling revealed a significant increase in the production of metabolites such as pipercolate and L-tryptophan. Additionally, increased temperature has an impact on the production of aminoacyl-tRNA and plant secondary metabolites (Thomason et al., 2018). Using LC-MS/MS-HPLC techniques, the metabolic characterization of bread wheat grains after heat stress revealed greater amounts of sucrose and G1p (Wang et al., 2018).

Concluding Remarks & Future Outlook

In modern biological research the field of metabolomics has accomplished a valuable place. Because it has ability to applicable in various fields of plant sciences like cell mechanisms and their responses to numerous stresses. In recent era, it is allowing to improve the key properties of plants quality, yield, shelf life by assessing their pathways. The integration of modern tools with omics approaches in bioinformatics adeptly analyze novel metabolic networks for crop betterment.

In future metabolic markers identifications can enhance the plant metabolic pathways detection. It can improve the capability of a breeder in order to plan and develop superior plants that adequately fulfil the modern era needs. It can be associate with speed breeding and like other approaches for rapid research purpose.

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