Metabolome Analyses in Response to Diverse Abiotic Stress

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Abstract

Advanced technologies, particularly “omics” approaches, include the most immediate and promising options for enhancing crop abiotic stress resistance. Omics approaches help discover the molecular mechanism to stress response and tolerance. The application of metabolite profiling techniques in the detection, unambiguous identification, quantification, and quick analysis of even minute quantities of cellular metabolites has risen dramatically in recent years. Metabolomics is essential for understanding plant chemical imprints at various stages of growth and development. The major issues that world agriculture faces today are feeding an ever-increasing population with finite inputs and in a rapidly changing environment. In this light, metabolic engineering technology appears to be a feasible choice, with enormous promise for delivering future crops. Proteomics and metabolomics investigations in cereals, in reaction to abiotic stress, are still negotiable. Gene manipulation involving regulatory genes for improved abiotic stress tolerance has proven to be more effective than employing single or multiple stress responsive genes. Recent breakthroughs in “omics” technologies and access to genomic sequences have a significant potential to

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improve stress tolerance in cereals. This chapter describes several methodologies to detect metabolites in response to abiotic stress in significant cereal crops.

Keywords

Cereals · Abiotic stress · Omics · Metabolomics

6.1 Introduction

Plants that are sessile in nature are driven to survive in harsh environments resulting from either physical stress (abiotic stress) or through other living species (biotic stress). Drought, salt, waterlogging, severe temperatures, and heavy metals are all abiotic stressors that limit plant growth and development, resulting in yield reductions of greater than 50% for important agricultural plants (Bray et al. 2000). Non-stress agricultural land accounts for only 10% of total arable area worldwide, suggesting that crops grown in the rest cultivable lands are prone to abiotic stresses (Dita et al. 2006). Furthermore, crops that are subjected to abiotic stresses are more susceptible to weeds, insects, and pathogen infections, resulting in significant losses (Reddy et al. 2004). The advancement of omics technology and functional analysis of individual genes has revealed that environmental acclimation, which is critical for plant survival, is tightly regulated (Kissoudis et al. 2014; Lopez et al. 2008). Abiotic stress factors like soil salinity and high temperatures, in addition to drought, have a huge impact on crop output. They cause yield losses that are variable in nature related to stress timing, intensity, and time duration. As a result, improving abiotic stress tolerance in crop plants is critical for increased yield stability.

6.2 Omics and Abiotic Stress Tolerance in Cereals

A thorough understanding of both the target crop species and the underlying stress tolerance processes, which includes measurements from the whole plant to the molecular level, is required for successful application of biotechnological techniques to overcome abiotic disturbances. The kind, duration, and severity of stress have a major impact on plant stress response (Bray 1997). Given that plant responses are dictated by the genome, current efforts have focused on deciphering the molecular response to abiotic stressors. Until recently, model plants like *Arabidopsis* and rice were the focus of plant stress response research, while orphan crops like millets languished far behind.

Omics is a term that refers to all biotechnological applications that require knowledge of stress response at the molecular level, such as genomics, functional genomics, genetic engineering, gene expression, protein or metabolite profile(s), and their overall phenotypic effects in response to environmental perturbations, which are usually accompanied by significant changes in the plant transcribing system (Ahuja et al. 2010). Cereal crops have recently benefited from “omics” techniques to better understand the molecular and genetic underpinnings of abiotic stress perception, response, and tolerance. Transcriptome and expression profiling investigations have been performed on a regular basis in the past. Abiotic stress research on major cereals has made significant progress so far, as detailed in the following sections.

6.3 Different Platforms to Gather Metabolomics Data

Because most research projects focus on understanding or improving a particular target feature, targeted metabolomics is by far the most prevalent method. There is a wealth of information available to explain phenotypic variance, but it may not be readily available. Small molecules have a big impact. Tomatoes, for example, can taste bitter, sweet, insipid, or wonderful depending on the ratio of sweetness to acidity (Baldwin et al. 2000). Large-scale, low-cost assays that can analyze hundreds of samples in a short amount of time are required to accelerate advancements through breeding programs.

Combining numerous datasets from complementary analytical platforms, on the other hand, provides a powerful technique for metabolome analysis. Color and aroma are two further areas where tomatoes can be improved. Isoprenoids, such as carotenoids, make up the majority of tomato pigments, while polyphenolics (such as flavonoids) make up the rest (Grotewold 2006). For carotenoid profiling, liquid chromatography (LC) with commercial standards has been employed in the past (Enissi et al. 2005). However, LC-MS is employed to provide more complete metabolome estimation, particularly for isoprenoids.

The quality (i.e., isomerization) and identity (i.e., heat degradation) of carotenoids can change throughout food preparation and processing. For a comprehensive description of isoprenoids, examination of both raw and cooked samples is required (Dewanto et al. 2002; Re et al. 2002). Carotenoids provide a variety of benefits in addition to color. Carotenoids, as well as fatty acid and amino acid derivatives, contribute to fruit aroma in addition to color (Tieman et al. 2006). All three are volatile chemicals, and they are separated and identified by GC and GC-MS (Tikunov et al. 2005).

One of the common issues encountered while examining MS or NMR data in order to better comprehend the spectra formed during an experiment is the need for a well-curated database. Recent advances in tomato metabolomics have resulted in the emergence of such community-oriented tools. Several software and analysis tools have been developed in recent years to process and evaluate metabolite data, but none of them is self-sufficient enough to meet user expectations.

6.4 Metabolomics

The term “metabolome” refers to the entire set of low-molecular-weight chemicals found in a sample that may be by-products or substrates of enzymatic activities. Furthermore, changes in the cell metabolome are not always related to changes in the proteome or transcriptome, underscoring the importance of metabolomics. Target analysis, fingerprinting, and metabolite profiling are some of the conceptual techniques in metabolomics that may be utilized for large-scale applications, such as phenotyping transgenics, gene function identification, significant equivalence testing, and stress response monitoring. As a result, metabolomics bridge the gap between genotype and phenotype. Most of the metabolomics studies used model plants and cereals, with no or very little studies on main millet crops. Only one study used gas chromatography, to investigate variation among primary metabolites and phenoli acids in three kinds of proso millet.

6.5 Metabolomics Intervention Toward Better Understanding of Plant Traits

The above techniques enabled the collection of metabolome data from samples taken for primary and secondary metabolite analysis, as well as lipidomics under varied growth conditions. The metabolomics research looked into biotic stress (Kumar et al. 2017; Uchida et al. 2020), abiotic stress (Feng et al. 2020), improving the quality of legumes and cereals (Tripathi et al. 2015; Okazaki and Saito 2016), biofuel production and lipid profiling (Hou et al. 2016; Tenenboim et al. 2016), the impact of climate change and high CO_2 levels (Pal et al. 2014; Reich et al. 2016), hormone profiling (Kumar et al. 2018), and improving fruit quality (Toubiana et al. 2012; Ainalidou et al. 2015; Upadhyaya et al. 2017). These efforts allowed researchers to examine metabolic pathways in order to build stress-tolerant and nutrient-dense crop plants (Kumar et al. 2017). Several review publications have previously focused on providing thorough methodology and the availability of advanced tools utilized in omics studies, including metabolomics (Kumar et al. 2017; Raja et al. 2017).

Metabolomics is a broad term that refers to the study, characterization, identification, detection, and quantification of metabolites from cells, tissues, and living organisms under specific environmental conditions (Parida et al. 2018; Kumar et al. 2016). The metabolome is made up of a large number of tiny molecules (lower than 2000 Da) with a wide range of chemical structure and makeup. In their analyses of endogenous metabolites and metabolites from external sources, the researchers use both non-targeted and targeted techniques (Li et al. 2014). In comparison to proteomics and transcriptomics, this method generates more detailed data (Dos Santos et al. 2017). The development of high-performance liquid chromatography (HPLC), mass spectrometry (LC-MS and GC-MS), NMR, direct injection mass spectrometry (DIMS), and other techniques has aided in the understanding of metabolite profiling and stress tolerance mechanisms in plants (Parida et al. 2018). Metabolomics studies have gained significance in plant science research due to their accuracy, sensitivity, and precision in mitigating agricultural losses (Genga et al. 2011) and revealing various facts about plant signaling and regulatory pathways (Shen et al. 2016). The overall metabolite content in plants is estimated to be around 250,000 (Kim et al. 2010). The overall quantity, concentration, and kinds of metabolites in plants are greatly increased under stress situations. Plant metabolite profiles show a clear correlation with changes in gene expression. It is critical to learn about the crucial metabolites that play a role in growth, development, and survival, as well as how they are modulated when diverse abiotic stresses occur. Various researchers (Freund and Hegeman 2017; Kumar et al. 2016) identified potential metabolomics markers that are critical for plant abiotic stress tolerance. Several researchers have employed the metabolomics approach to investigate metabolic patterns in stressed plants (Yan et al. 2014; Muthuramalingam et al. 2018; Shen et al. 2016; Srivastava et al. 2013; Witt et al. 2012). As a result, it has become a crucial tool for deciphering the molecular pathways that underpin stress responses. Drought stress caused the increase of many metabolites in *Arabidopsis thaliana* plants, including proline, raffinose like oligosaccharides, GABA, and several tricarboxylic acid (TCA) cycle metabolites (Urano et al. 2009). The function of various subunits of the mitochondrial alternative electron transport route was changed under dark stress (Araujo et al. 2011). Abiotic stress, such as salt, drought, and other factors, also increased the amounts of branched-chain amino acids (BCAAs). The amount of amino acids accumulated is determined by the degree of desiccation, as evident from amino acid profiling of maize and wheat during water desiccation (Witt et al. 2012). Colmsee et al. (2012), for example, created the OPTIMAS-DW data resource platform to solve various concerns about *Zea mays*. It can be used to integrate metabolomics, transcriptomics, proteomics, and ionomics data as well as handle different data domains. Amiour et al. (2012) identified important regulatory processes in nitrogen metabolism control by combining metabolomics, proteomics, and transcriptomics research.

Srivastava et al. (2013) reported on a study involving a transgenic plant that included the *superoxide dismutase* (*SOD*) gene. They used a data processing platform to generate information on ROS metabolism at the system level. Yan et al. (2014) concentrated on the use of omics techniques to better understand secondary metabolism. Exposing plants to salt stress, AbdElgawad et al. (2015) found that tocopherol levels in maize shoots increased, but ascorbic acid levels plummeted. Wang et al. (2015) also found that when *Kosteletzkya virginica* seedlings were exposed to salt conditions, their proline levels increased. Under salt stress, Shen et al. (2016) found a quick decline in the levels of sugars associated with glycolysis pathway in barley. When the peach plant was subjected to higher temperature, it was found to accumulate proline. Sun et al. (2016) examined how the metabolome of maize changed after being exposed to diverse stresses such as drought, heat, and salinity. Based on the metabolomics data, they found that the individual stresses affect differently from that arising from a mixture of stresses. Khan et al. (2018) used untargeted metabolic profiling techniques to examine the influence of drought on the metabolome of chickpea genotypes. Muthuramalingam et al. (2018) investigated threonine profiling using genome-wide computational metabolomics. Using in silico expression investigations, they discovered roughly 16 genes that regulate threonine levels in abiotically stressed rice plants.

Plants create an ample amount of metabolites, having a variety of structures and essential for plant growth and development. These metabolites lay the chemical foundation of agricultural output and quality, as well as an important source of nutrition and energy for humans and livestock (Hall et al. 2008). These metabolites could be primary and secondary; primary metabolites are requisite for plant growth, while secondary metabolites do not have a role in growth, but required for a plant to survive in stressful situations by maintaining a balance with the environment. Exploration of the underlying biochemical nature of plant metabolites and their function in complex regulatory mechanisms is necessitated by the diversity of plant metabolites and their role in intricate regulatory mechanisms (Hall et al. 2008). Because of the complexity of the varied metabolic properties and abundances of chemicals, studying the metabolome of plants will be extremely difficult. Various abiotic stressors disrupt plant metabolism. Under stress, plant metabolic networks must be reconfigured to allow for maintaining the metabolic homeostasis as well as the generation of chemicals that alleviate the stress. Plant development and productivity are reduced when they are exposed to unfavorable growing conditions, such as abiotic stress. Plant metabolism is disrupted for a variety of causes, including inhibition of metabolic enzymes, a lack of substrate, an excess demand for certain chemicals, or a combination of these and other variables. As a result, the metabolic network must be reorganized so that essential metabolism is preserved and a new state for adaptation to the current stress conditions is adopted.

Another issue is the generation of ROS, causing oxidation that disturbs the function of biological components and, in the worst-case scenario, cell death. To reduce ROS formation, the reducing power to scavenge ROS is provided via organellar electron transport chain and must be well maintained (Das and Roychoudhury 2014). Despite the importance of metabolic control in stressful situations, our knowledge of the process is still fragmented and incomplete. As a result, metabolomics is used to gather a huge number of useful information for gene discovery and pathways (Tohge et al. 2014). Plants appear to have a complex regulatory network covering these small molecules, and metabolomics research contributes greatly to understand the relationship between genotype and metabolic outputs by targeting important network components and finding interactions among these metabolites (Toubiana et al. 2013). The domains of transcriptomics, genomics, proteomics, and metabolomics have all seen significant advancements during the "Omics" era. The data collected by these "Omics" methodologies has improved the precision and speed with which breeding programs are generating nutrition-rich and climate-smart germplasm to ensure food security (Parry and Hawkesford 2012). In recent years, the importance of phenomics-based breeding has become clearer.

Based on the technique, metabolomics can be divided into two categories: untargeted metabolomics and targeted metabolomics. Untargeted metabolomics refer to comparing the metabolomes of control and test groups in order to uncover changes in metabolite profiles that may be related to specific biological circumstances. Targeted metabolomics acts as a quantitative method for identifying and analyzing specific metabolic components in living organisms. It provides details on the amount and composition of metabolites, which are directly linked to biological functions and can vary substantially depending on physiological conditions. As a result, metabolomics techniques are critical for understanding physiological functions of various organisms. Metabolomics is a strong method for gaining a thorough understanding of how metabolic networks are regulated, and it has been used in a number of studies in recent years.

Plant plasticity can also be better understood for future biotechnological engineering of stress-tolerant plants. In spite of the fact that it is currently impossible to perform an accurate and thorough full metabolome analysis of a sample, plant metabolomics approaches and technology are quickly evolving (Hegeman 2010). A variety of integrated technologies, such as non-destructive NMR, mass spectrometry (GC-MS and LC-MS), and FI-ICR-MS (Fourier transform ion cyclotography-MS), have made possible large-scale analysis. Metabolomics could be carried out at the subcellular level with the help of various sample technologies (Misra et al. 2014). A number of common plant species, like tomato, wheat, rice, and maize, have proved their potential in plant metabolomics investigations for many reasons (Francki et al. 2016). However, because each analytical platform has inherent limitations, combination techniques are increasingly being used in metabolomics investigation.

6.5.1 GC-MS

The most widely utilized technology for plant metabolomics research is gas chromatography-mass spectrometry (GC-MS). Before separating them through GC, polar metabolites are being derivatized to make them volatile. The fact that this technology has been used for metabolite profiling for a long time means that there are established methods for chromatogram evaluation and interpretation (Lisec et al. 2006). The short run time and cheap running cost of GC-MS make it more significant, but the technique can only be applied for thermally stable volatile chemicals, making analysis of high-molecular-weight molecules (more than 1 kDa) challenging.

6.5.2 LC-MS

In this technique, a prior sample treatment is not required, and separation of the components occurs in a liquid phase, thus not limited by compound volatilization. Reversed phase, hydrophobic interaction, and ion exchange columns are among the columns that can be used to separate metabolites depending on their chemical features, making the technique applicable to a wide range of plant metabolites. The recent development of ultra-performance liquid chromatography (UPLC), with higher resolution and sensitivity, makes the approach more potent. For ionization to connect LC and MS, electrospray ionization (ESI) is extensively utilized.

6.5.3 NMR

Nuclear magnetic resonance spectroscopy is a completely different type of analytical technique than MS, which is based on atomic interaction. Atoms with nonzero magnetic moments, such as 1H, 13C, 14N, and 15N absorb and reflect electromagnetic radiation in a strong magnetic field. The intensity, frequency, and magnetic relaxation qualities of the radiation are all characteristics that reveal the particular environment of the identified nucleus. As a result, the atoms of a molecule produce a unique spectrum that can be used to identify and quantify metabolites in a complicated biological sample. NMR can also create in vivo kinetic data and investigate metabolic reactions in a single plant, rather than a group of similar plants (Terskikh et al. 2005). The magnetic characteristics of nuclei in a magnetic field are used to detect metabolites via NMR. NMR is a nondestructive approach for identifying metabolites with a molecular weight of less than 50 kDa for a variety of applications, including metabolite fingerprinting, metabolic flux, profiling, and extracting the atomic structure information of compounds in biological materials (Winning et al. 2009). MS has improved its accuracy because of the advancements in ionization technologies like electrospray ionization (ESI), air pressure chemical ionization (APCI), and MALDI-TOF (Issaq et al. 2009). Metabolomics is becoming more prevalent in plant sciences and biochemistry, and it has been used in a huge number of researches to date. In this chapter, we shall look at how metabolomics profiling can be used to figure out why plants are so resilient to abiotic stress.

6.6 Abiotic Stresses

6.6.1 Water Stress

Water scarcity is one of the most serious risks to crop output, and it is expected to worsen significantly in the future years (Cominelli et al. 2009). As a result, a lot of research has gone into understanding how people react to this important and prevalent stress. These investigations have indicated that metabolic regulation, particularly photosynthetic regulation and osmolyte buildup, plays a vital role in drought stress response (Verslues and Juenger 2011; Rabara et al. 2021). Plants respond to drought stress by accumulating several metabolites (Urano et al. 2010). The principal metabolites that changed in abundance in response to water deficiency in wheat were amino acids, organic acids, and sugars. Polyamine production is linked to tryptophan build-up via the shikimate pathway and glutamate reduction. Non-targeted metabolomics data analysis revealed that the biomolecules and secondary metabolites were differentially accumulated in the leaves in response to drought and heat stress. Excess water, such as flooding or rhizosphere waterlogging, creates issues due to a reduction in oxygen supply (hypoxia/anoxia). Fermentation in anoxic conditions is required to create ATP, resulting in cytosolic acidification and the build-up of hazardous chemicals. There is a large-scale metabolism reprogramming under oxygen-reduced conditions, which was supported by transcriptome data indicating a widespread downregulation of energy-consuming activities (van Dongen et al. 2009).

6.6.2 Temperature

Cells are severely affected due to ice formation and cellular membrane malfunction (Guy 1990) due to freezing. The molecular foundation of the cold acclimation process has been thoroughly investigated, as has the role of certain metabolites, such as compatible solutes (Wanner and Junttila 1999), as well as the regulatory network (Medina et al. 2011). Cook et al. (2004) and Kaplan et al. (2004) observed that the bulk of heat shock reactions, such as increased pool sizes of amino acids, polyamine precursors, and suitable solutes, were shared between heat stress and cold shock. The findings of this work were compared to transcript profiling data (Kaplan et al. 2007), which demonstrated that transcriptional and posttranscriptional mechanisms regulate GABA shunt and proline buildup under cold circumstances, respectively.

6.6.3 Light

Light drives photosynthesis and also causes secondary damaging processes during excess irradiance. Wulff-Zottele et al. (2010) measured metabolites in plant leaves for 6 days after switching to high light. The level of most glycolysis, Kreb cycle, and pentose phosphate pathway metabolites was altered, showing that the plants exposed to high light undergo a metabolic shift. In addition, an increase in glycine suggested that photorespiratory pathway was activated. Metabolites like glycine and glycolate, which are photorespiratory intermediates, were observed to be accumulated in the early period (5–60 min after changeover). Kusano et al. (2011) observed that the metabolic effect of UV light stress on plants during the mid-phase (80–360 min) embraces the buildup of phenylalanine, shikimate, and fructose, plus the decrease of succinate.

6.6.4 Ion Stress

High salinity levels in the soil impede crop growth, posing severe issues for global food production (Munns 2005; Roychoudhury et al. 2008). High NaCl concentration generates both hyperionic and hyperosmotic stress in plant cells, resulting in turgor loss, disrupted metabolism, and suppression of critical ion absorption, as well as other issues (Kim et al. 2007; Roychoudhury et al. 2007). The metabolite concentrations were frequently many times greater in *Thellungiella*, and stress aggravated the condition. Some metabolites differ from one another. The metabolic differences between *Arabidopsis* and other plants and *Thellungiella* were tested for a wider spectrum of salt and osmotic stressors (Lugan et al. 2010). The examination of global physicochemical parameters of both the species revealed several metabolites, but the shift in *Thellungiella* was far more dramatic. Heavy metals, in general, cause cellular oxidation, enzyme inhibition, and metabolic disturbance, which results in retarded growth and, in extreme cases, mortality (Sharma and Dietz 2009). Dubey et al. (2010) performed a transcriptomics and metabolomics investigation of rice roots treated with Cr. Proline, as well as ornithine, accumulated at three times the rate of the control under these conditions. Following Cr stress, the concentration of various additional metabolites such as lactate, fructose, uracil, and alanine increased. The findings showed that control of sucrose degradation was acting as a rescue mechanism when respiration was stopped.

6.6.5 Combined Stress

In nature, adverse environmental conditions are frequently the result of a combination of variables, with one stress usually being followed by another (Králová et al. 2012). Plants are frequently exposed to multiple stresses, each of which is followed by another. The single stress level is most convenient for experimentation and discussion; however, the plants are actually exposed to a variety of abiotic stress in their habitat (Paul and Roychoudhury 2019). When maize plants are subjected to both drought and salinity stress, levels of citrate, fumarate, valine, phenylalanine, isoleucine, and leucine levels in leaves alter dramatically, indicating a crosstalk effect in manifold stresses (Sun et al. 2015). Combined cold and water stressors in rice resulted in overexpression of carbohydrate metabolism-related genes, which is consistent with the accumulation of glucose, sucrose, and fructose in the aerial portions (Maruyama et al. 2014). When plants are subjected to combined stressors, sugars typically accumulate in plant cells, possibly buffering plants from oxidative damage via osmotic adjustment (Wulff-Zottele et al. 2010).

6.7 Conclusion

Molecular biology and biotechnology have emerged as a viable method for combating stressors in plants in recent years. However, knowledge of stress response at the molecular level is required for efficient application of omics for abiotic stress tolerance. High-throughput sequencing technologies are capable of providing researchers with a wide range of sequencing applications, including the detection of short RNAs and SNPs and the finding of molecular markers. NGS techniques, in combination with genome-wide expression profiling studies, will overcome the challenges posed by large genomes, particularly those found in cereal grains. Proteomics and metabolomics investigations in cereal crops are still lagging behind genomic and transcriptomics studies. However, advances in high-throughput proteomics and metabolomics tools like time-of-flight mass spectrometry and flow injection will simplify the study on complex biological systems like cereals. It not only will help in identifying genes, proteins, and metabolites responsible for many molecular and signaling pathways but will facilitate our fundamental understanding of the molecular basis of abiotic stress tolerance in such crops. As a result, it is critical to carry out considerable research in cereals to overcome abiotic stress. A collaborative effort involving all omics will be critical in unraveling abiotic stress tolerance mechanisms, which may then be used for MAS or traditional breeding. QTLs in many cereals are yet undetected, with no indications of gene pyramiding. In addition, rigorous and meaningful phenotyping is necessary for understanding stress adaptation mechanisms before locating and tagging genes. Nonetheless, current improvements in omics technologies, as well as advances in transgenic technology and MAS, will help improve the existing situation, and it will be possible to genetically modify all cereals in the future.

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