CHAPTER

COMPARATIVE METABOLOMICS APPROACH TOWARDS UNDERSTANDING CHEMICAL VARIATION IN RICE UNDER ABIOTIC STRESS

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26.1 INTRODUCTION

Cereals such as rice, wheat, barley, corn, etc. are the major food crops grown worldwide. Rice (*Oryza sativa* L.-Poaceae), with a genome size of 430 Mb is an experimental model plant for understanding the biology of all cereals (Manavalan et al., 2012) and also provides food for a large part of the world's population as a part of their staple diets, especially in East and South Asia, the Middle East, Latin America, and the West Indies. Rice is grown in a diverse range of environmental conditions characterized by different temperatures, climates, and soil—water. It can grow in areas intolerant to other crops. More than 400,000 rice germplasm accessions are stored in gene banks around the world (Toriyama et al., 2005), suggesting that the chemical diversity across rice varieties is large (Kusano et al., 2014). Adverse environmental conditions seriously threaten rice production and cause enormous losses worldwide, even in the most productive irrigated lands. Abiotic stress is a major factor limiting productivity of crop plants in large areas of the world. Abiotic stresses such as salinity, water availability (less or excess water), temperature extremes (freezing, cold, or high), metal/metalloids, nutrient stress, etc., all represent a serious threat to sustainable rice production (Fig. 26.1). The degree of losses due to abiotic stress varies and depends on the intensity and duration of the stress. Metabolomics has contributed to the identification of metabolite biomarkers that are associated with the tolerance of rice plants to stress, particularly abiotic stress.

Stresses are complex and multigenic traits that affect plant performance, and in order to counteract the adverse effect of environmental assaults, plants have evolved efficient defense mechanisms by manipulating their tolerance potential through integrated molecular and cellular responses. In general, the defense machinery involves the activation of stress-inducible genes and their products—that is, secondary metabolites, volatile or bioactive compounds for example, antioxidants and phytohormones for example, abscisic acid (ABA) (Kusano et al., 2014), which are either

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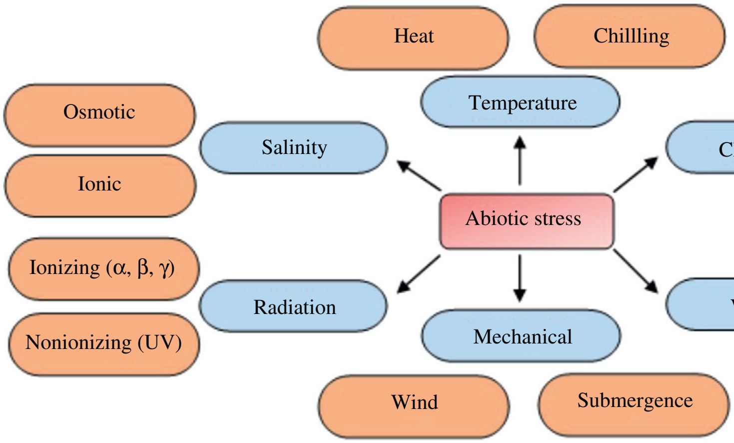

FIGURE 26.1

Schematic diagram showing different abiotic stresses.

functional or regulatory in nature to ascertain direct stress tolerance or through the downstream signal transduction pathway. Therefore, it is desirable to understand the physiological and molecular aspects of plant functions under stressful conditions, for example, the activation of cascades of molecular networks (perception of stress signals, transducers, transcription regulators, target stress-related genes, and metabolites). Comparative metabolomics approach toward understanding the abiotic stress to plants thus reflect the integration of gene expression, protein interaction, and other different regulatory processes and are, therefore, closer to the phenotype than mRNA transcripts or proteins alone. Rice metabolomics involves the study of both qualitative and quantitative analysis of both primary and secondary metabolites (with molecular weight up to 1500 Da) of rice plants during its developmental stages (Khakimov et al., 2014). It offers an insight into the metabolic fluctuations of different rice cultivars that may reveal the effects of genetic modifications as well as of biotic and abiotic stress (Khakimov et al., 2014). Metabolomics in its entirety serves as the biochemical snapshot of an organism's phenotype (Kusano et al., 2011). It is also a promising approach to reveal the biochemical and genetic backgrounds of quality traits and may open new possibilities toward targeted breeding (Fernie and Schauer, 2009; Bino et al., 2004).

To date, most research has focused on identifying genes associated with abiotic stress tolerance in rice by comparing the gene expression profiles of certain tolerant and sensitive genotypes (Kawasaki et al., 2001; Walia et al., 2005; Cotsaftis et al., 2012). Metabolites are the final products of various regulatory processes such as transcriptional, post-transcriptional, translational, post-translational, and allosteric regulation, etc., and therefore, comparative metabolome in response to stress could more closely reflect the molecular phenotype of the plant rather than the transcript or protein levels, thus, offering better insights into the biological mechanisms that impart stress tolerance abilities (Arbona et al., 2013). Global unbiased metabolite profiling in crop plants exposed to various abiotic stresses are almost unknown, and so far only a few studies of stress-associated metabolome have been reported in rice (Fig. 26.2). For example, comparative metabolomics of M2O2

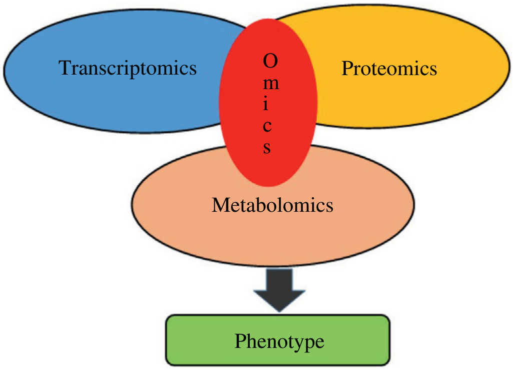

FIGURE 26.2

Schematic diagram showing amalgamation of omics in relation to specific phenotypes.

(submergence-sensitive) and M2O2Sub1 (tolerant) rice genotypes revealed significant differences in amino acids (AAs), tricarboxylic acid (TCA) cycle intermediates, organic acids (OAs), and myo-inositol and trehalose accumulations (Barding et al., 2012).

The studies of the comprehensive metabolic approach require a reliable technique and protocol that enables extraction of maximum metabolic information in a high-throughput and reproducible manner. Using metabolomics to study the effects of different abiotic stresses in crop plants, hinders the approach for uncovering single or multiple internal and/or external effects due to the great physico-chemical diversity of the crop metabolites. The estimation of the chemical structure(s) of unknown metabolites in metabolite profile data continues to present challenges (Nakabayashi and Saito, 2013). Metabolomics employs a range of analytical technologies including nuclear magnetic resonance (NMR) based techniques and mass spectrometry (MS), the most common of which is GC−MS which allows rapid, non-biased, and simultaneous analysis of a large number of small molecules. The development of comprehensive metabolomic techniques (such as ultrahigh-resolution MS e.g., LC−MS combined with Fourier Transform ion Cyclotron Resonance), biochemical database (MetaCyc), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) under different conditions, have helped in the non-targeted metabolic profiling and identification of different metabolites and increased the possibility of precisely estimating the molecular formula of the peaks of unknown metabolites (Caspi et al., 2018; Kanehisa et al., 2012; Morreel et al., 2014). RiceCyc is a rice-specific metabolic pathway database constructed based on MetaCyc and plant metabolic network (Chae et al., 2007).

26.2 RICE METABOLOMICS

Metabolomics has been applied to investigate the response of rice to abiotic or biotic stress. Metabolites (e.g., phenolics or OAs, etc.,) vary in response to different abiotic stresses. Various metabolites are developed in response to stress as a mechanism of tolerance or a change in normal metabolomic pathways. Therefore, untargeted metabolite profiling is often used in metabolomic

Table 26.1 Metabolomics Research in Rice Against Abiotic Stress (Okazaki and Saito, 2017)

Sl. No.	Research Materials	Analytical Method	Analytes	References
1	Leaves of rice challenged with submergence	^{1}H NMR	Mainly primary metabolites	Barding et al. (2012)
2	Developing caryopses grown under high night temperatures	CE−MS	Primary metabolites	Yamakawa and Hakata (2010)
3	Leaves of rice cultivars grown under high night temperatures	GC−MS	Primary metabolites	Glaubitz et al. (2015)
4	Floral organs of rice cultivars under heat stress	GC−MS	Primary metabolites	Li et al. (2015)
5	Leaves of rice challenged with drought stress	GC−MS	Mainly primary metabolites	Degenkolbe et al. (2013)
6	Aerial parts of rice treated with cold and drought stress	GC−MS, LC−MS, CE−MS	Mainly primary metabolites	Maruyama et al. (2014)
7	Rice challenged with salt stress	GC−MS	Primary metabolites	Zuther et al. (2007)
8	Leaves of rice treated with ozone	CE−MS	Primary metabolites	Cho et al. (2008)
9	Submerged rice leaves at different times	^{1}H NMR	Both primary and secondary metabolites	Barding et al. (2012); Jung et al. (2010)

studies. It is particularly useful to obtain a global view of the metabolism of cells or identifying new metabolites/pathways developed in response to stress. The metabolome profile is very sensitive to abiotic stress. Metabolomics allows the investigation of the genetic background of quality traits in rice, the metabolic changes triggered by light and dark cycles, and biomarkers that represent the developmental period of rice plants. Some of the analytic methods along with the techniques used in rice have been summarized in Table 26.1.

26.3 METABOLIC CHANGES IN RICE UNDER DIFFERENT ABIOTIC STRESSES

26.3.1 SALT STRESS

High salinity is when salt stress triggers both hyper-osmotic stress (caused by the reduction of water availability due to the reduced water potential) and hyper-ionic stress (caused by the toxic effects of the accumulated ions). Plants are, thus, subjected to dehydration, ion toxicity, nutritional deficiencies, and oxidative stress, with the main negative effects being the disruption of ionic equilibrium, the inhibition of cell division and expansion, and the reduction in photosynthesis and growth. Rice acclimation responses include ion exclusion, osmotic adjustment, tissue tolerance, and several molecular and biochemical changes with both conserved and divergent metabolic responses among different species (Sanchez et al., 2008). Soil salinity is one of the major constraints affecting rice production worldwide, especially in coastal areas. Rice (termed a glycophyte) is very sensitive to salinity specifically at the early vegetative and later reproductive stages, with rice varieties differing remarkably in salt tolerance. The metabolites were temporally, tissue-specifically, and genotype-dependently regulated under salt stress. Sugars and AAs increased significantly in the leaves and roots of both genotypes, while OAs increased in roots and decreased in leaves. Rice experienced greater increases in sugars and AAs and more pronounced decreases in OAs in both tissues, additionally, the maximum change in sugars and AAs occurred later, while OAs changed earlier. Such metabolites also known as osmoprotectants are considered as a basic strategy for the protection of crop plants from salinity and contribute to the maintenance of cytoplasmic water potential. AAs such as lysine, valine, proline, isoleucine, and threonine consistently increased in tolerant rice cultivars during salt treatment, whereas these same AAs were decreased or unchanged in sensitive rice cultivars. Sugar concentrations increased under salt treatment, indicating increased sugar is beneficial to salt tolerance. Sugar concentration between tolerant and sensitive variety vary with time variation. Sugars (sucrose, lactose, sorbitol, and mannitol) were consistently increased over the course of the stress treatment in tolerant cultivars. OA decreased under salt stress in both tolerant and sensitive and it varied in roots (more) and shoots (less). OAs such as fumaric acid, succinic acid, malic acid, and oxalic acid involved in the TCA cycle, decreased significantly in tolerant but increased or had no change in sensitive cultivars (Zhao et al., 2014). Moreover, less Na^+ and higher relative water content was observed in rice.

26.3.2 TOXIC METAL/METALLOID STRESS

Metals or metalloids, like chromium (Cr), aluminum (Al), cadmium (Cd), zinc (Zn), iron (Fe), lead (Pb), and arsenic (As) are highly toxic and reactive to living cells. Some toxic metals like copper (Cu), Zn, and Fe are essential micronutrients involved in various physiological processes but all become toxic above certain threshold concentrations. Heavy metal toxicity comprises of the inactivation of biomolecules either by blocking essential functional groups or by displacement of essential metal ions. In response to toxic levels of heavy metals, plants synthesize Cys-rich metal binding peptides including phytochelatins, metallothioneins, and various membrane transport systems play an important role in metal ion homeostasis and tolerance (Hall and Williams, 2003). Gene expression patterns change when plants encounter excessive amounts of toxic metals leading to changes in metabolite development. In rice crops grown in reduced environmental conditions, the toxic metals are solubilized in the soil solution, thus, risking uptake by the crop. Roots are the main organ for toxic metal penetration, thus, the roots of crop plants release metabolites such as OAs which bind to the metals to form insoluble complexes. OAs such as citric acid and malic acid act as ligands for metals (Rauser, 1995). The role of citric acid in regulating Al toxicity has been clearly demonstrated (Yang et al., 2012). Some of the effects of the toxic metals in rice are discussed here.

26.3.2.1 Arsenic toxicity

Arsenic (As) is a non-essential metalloid and is generally toxic to plants. This metalloid inhibits root extension and proliferation, and on translocation to the shoots severely inhibits plant growth by slowing or arresting expansion and biomass accumulation, thus, affecting the yield (Garg and Singla, 2011). At high concentrations, As interferes with critical metabolic processes, which can lead to the death of the plant (Finnegan and Chen, 2012). The ability of As(V) to be substituted for

Pi and the propensity of As(III) based compounds to bind to and change the activity of enzymes as well as the damaging effects of ROS all have direct and important consequences for plant metabolism. The plants response to these factors predominantly affects carbon, nitrogen, and sulfur metabolisms (Finnegan and Chen, 2012). As stimulates accumulation of ascorbate (Srivastava et al., 2005; Singh et al., 2006) and it appears that As does not have strong effects on gene expression related to the carbon metabolism. The NAD^+ formate dehydrogenase protein accumulates in the leaves of rice plants exposed to As(V) (Ahsan et al., 2010). In As(V) treated rice, the amount of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein decreased in the roots and increased in the shoots (Ahsan et al., 2008; 2010). The roots of As(V) treated rice plants had decreased amounts of transcripts for NO^{3-} transporters and for NH^{4+} transporters (Norton et al., 2008).

26.3.2.2 *Chromium toxicity*

Cr(VI) is phytotoxic either at any concentration or above a certain threshold level (Nieboer and Richardson, 1980). It results in the inhibition of seed germination, nutrient balance, antioxidant enzymes, and induces oxidative stress in crop plants (Panda, 2003). Under Cr(VI) stress, total chlorophyll content decreased and carotenoid content, which act as an antioxidant to scavenge ROS generated as a result of Cr toxicity, increased (Panda and Choudhury, 2005). Significant increase in lipid peroxidation and free proline levels were observed in response to Cr toxicity. Rapid accumulation of free proline is a typical response to toxic metal stress.

26.3.2.3 *Aluminum toxicity*

Under aluminum (Al^{3+}) toxicity, in acidic conditions, various changes in cellular metabolic activities occur (Awasthi et al., 2017) such as increased ROS, leading to the oxidation of biological macromolecules and, as a consequence, to lipid peroxidation, membrane damage, and enzyme inactivation. To alleviate oxidative injury, non-enzymatic systems [such as reduced glutathione (GSH), ascorbic acid (AsA), carotenoids, and phenolic] and enzymatic systems such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), glutathione reductase (GR), and glutathione peroxidase (GPX) are activated (Ma et al., 1997). Aluminum (Al) stress generates ROS in rice plants and consequently increases the levels of ROS scavenging proteins (Dubey and Sharma, 2007; Pandey et al., 2013). Metabolites such as OAs (citrate, malate, oxalate, etc.,) released in the activation of various Al induced genes (e.g., ALMT1and MATE) lead to tolerance against Al toxicity. Al is bound to oxalate ions in the roots and leaves and to citrate ions in the xylem (Ma et al., 1997; Zheng, 1998) ameliorating toxicity to the crop.

26.3.3 DROUGHT STRESS

Drought is one of the major abiotic stresses limiting to plant production. Droughts can be the result of an overall decline in rainfall in wet or dry season, a shift in the timing of the wet season, or a strong local warming that exhausts water bodies and soils through evaporation (World Bank, 2013). Rice is particularly susceptible to soil—water deficit. For upland rice, drought is a major constraint on productivity (Witcombe et al., 2008) and for rainfed lowland rice drought is the main environmental factor reducing productivity, by up to 35% (Jongdee et al., 1998). Drought delays the development of rice plants, and strongly affects the morphological as well as physiological processes

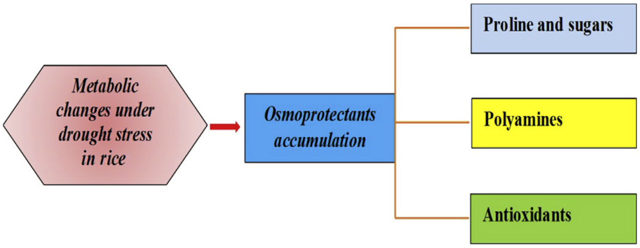

FIGURE 26.3

Schematic diagram showing accumulation of different metabolites under drought.

like transpiration, photosynthesis, respiration, and translocation of assimilates to the grains. Plants display a wide range of mechanisms for withstanding drought stress (Nahar et al., 2016; Sarma et al., 2016). Rice shoots are metabolically deactivated during drought to reduce the consumption of water and nutrients, whereas roots are metabolically activated to enhance the uptake of water and nutrients, together buffering the effects of drought. Metabolites such as low molecular weight osmolytes, including glycine betaine, proline and other AAs, OAs, and polyols, are crucial for crop plants to sustain cellular functions under drought conditions. The increase in drought stress was found proportional to drought resistance levels that is, the increase in stress leads to the increase in metabolites in relation to the defense function. Under stress, some metabolites are co-related to sensitivity and tolerance. Many AAs (asparagine, glutamate, glutamine, glycine, serine, and threonine) showed high concentrations in drought-sensitive cultivars with low biomass under drought stress. Thus, high AA levels observed in sensitive cultivars reflect the increase in protein degradation and the decrease in protein synthesis under drought stress. At the physiological level the consequences of drought stress are very much reflective in the vital plant functionaries (metabolites or proteins) with the up- or down-regulation of various genes (Blum, 2011). The accumulation of soluble sugars, increase proline content, and increase in antioxidant activity have been the major mechanisms toward defense against drought stress (Fig. 26.3). A common effect of drought stress is the disturbance between the generation and quenching of ROS (Smirnoff, 1998; Faize et al., 2011). The enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase(GPX), enzymes of the ascorbate-glutathione cycle, ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (Noctor and Foyer, 1998). These antioxidants are critical components of the ROS scavenging system in plants, and their expressions can improve drought tolerance in rice (Wang et al., 2005). ABA, a phytohormone that regulates plant growth and development, rapidly increased under drought and saline stress (Mahajan and Tuteja, 2010). Shi et al., 2014 observed that various ABA levels in rice roots stimulate distinct ABA perception and signaling cascades, which influence the response to water stress.

26.3.4 HEAT STRESS

Heat stress is one of the biggest concerns resulting from the present climate changes. Increases in temperature can cause irreversible damage to plant growth and development (Wahid et al., 2007).

Baker et al. (1992) and Matthews (1997) reported a rice yield reduction of 7%–8% for each 1°C increase in day time temperature from 28 to 34°C. The rice plant appears to be most sensitive to high temperatures at flowering. Peng et al. (2004) and Mohammed and Tarpley (2009) found a negative correlation between increased night temperatures and rice yield. Since the majority of global rice is grown in tropical and semi-tropical regions, it is likely that higher temperatures would negatively affect rice production in these areas due to an increase in floret sterility that would subsequently decrease yields (Prasad et al., 2006). Increased temperatures will also affect the grain quality of the rice mainly through increased chalk content (Wassmann and Dobermann, 2007). Lisle et al. (2000) reported a higher number of chalky grains in rice grown in a glass house at 38/21°C compared to rice grown at 26/15°C, day/night temperature. Krishnan et al. (2011) presented that high-temperature stress causes loose packing of the amyloplasts, resulting in the formation of chalky grains.

26.3.5 COLD STRESS

Cold stress, which can be classified as chilling (0–15°C) and freezing (<0°C) stress, is a major environmental factor limiting the growth, productivity, and geographical distribution of crops. Rice, based on its origins being from tropical and subtropical regions, is considered more sensitive to cold stress than other cereals (Zhang et al., 2014). Cold stress affects chlorophyll content and fluorescence, and thus, interferes with photosynthesis in rice (Kanneganti and Gupta, 2008) and increased contents of ROS and malondialdehyde (MDA) which accumulate during cold stress impair the normal metabolism. Metabolites develop in response to the expression of stress-inducible genes and impart tolerance or defense to the crop plants against the effects of the stress. For example, cold-treated rice plants accumulate proline, an AA that stabilizes protein synthesis, and thereby, maintains the optimal function of rice cells (Kandpal and Rao, 1985). Under cold stress, the contents of antioxidant species also increase to scavenge ROS and protect rice plants against oxidative damage (Sato et al., 2011). The increase in the concentration of some metabolites under stress also serves as a marker for the evaluation of the plant against the stress and, thus, develop the plant with an increased tolerance capacity. Increase in concentrations or amounts of ROS, MDA, sucrose, lipid peroxidation, proline etc. Under cold stress, various genes were expressed leading to changes in metabolic pathways and variations in metabolites, which caused either positive or negative impacts on the tolerance of plants to stress (Zhang et al., 2014). Some of the genes involved; OsSAP8, TERF2 (Kanneganti and Gupta, 2008; Tian et al., 2011) led to changes in the chlorophyll content showing the positive effects of tolerance. OsAPXa (Sato et al., 2011), OsMKK6, OsMPK3, and OsNAC6 (Xie et al., 2012; Xu et al., 2011) showed an increase in superoxide radicals and MDA causing negative effects on plant tolerance. OSINV4, OsDREB1A (Tian et al., 2011; Oliver et al., 2005), OsDREB1A, and TERF2 (Tian et al., 2011; Ito et al., 2006) showed increased concentrations of sucrose, glucose, raffinose, and hexose which served positive effects on tolerance. The genes associated with the cold related AA, proline, such as OsMYB2, OVP1, OsNAC5, MYB4, OsPRP3, ZFP245, and OsMYB3R (Huang et al., 2009; Tian et al., 2011; Song et al., 2011; Zhang et al., 2011; Yang et al., 2012; Vannini et al., 2004; Gothandam et al., 2010), expressed in presence of cold stress showed an increase in concentration. Therefore, the expression of such genes shows a co-relation in the metabolic variation of the crop under cold stress.

26.4 CHEMICAL DIVERSITY IN RICE BASED ON METABOLITE UNDER ABIOTIC STRESSES

Rice is an excellent source of energy intake for about half of the worlds population. Due to climate change and global warming, the traditional rice landraces are under stress that delimit their growth and productivity. To determine the number of metabolites in rice and their chemical diversity under various abiotic stresses, the metabolite composition of cultivated rice need to be investigated with analytical techniques *viz*., MS and/or NMR spectroscopy and rice metabolite databases need to be constructed. Chemical diversity in rice based on metabolite under abiotic stress include sugars, amino and OAs, aromatic compounds, and phytohormones, which could be detected by either GC−MS, LC−MS, or CE−MS, etc. In a study conducted by Nam et al. (2015), the accumulation of sucrose, allantoin, glutamate, and threonine in salt-stressed rice was observed and this suggested that metabolites are likely to be salt stress markers that may be conserved in most of the genotypes, and may be closely related to the salt stress and/or adaptation response of rice roots. In rice roots exposed to long-term mild salinity, sucrose accumulation was found to be conserved as a metabolic response (Nam et al., 2015). Gupta and De (2017) explained that the metabolomics analysis of rice under salinity stress revealed elevation of serotonin, and gentisic acid levels in the leaves of tolerant varieties.

26.5 CONCLUSION

In this chapter, we discussed the various types of metabolites in rice in general and under abiotic stress in particular with an emphasis on the chemical diversity that includes primary metabolites, volatiles, phytohormones, lipids, and other secondary metabolites such as phenolics, flavonols, carotenoids, and alkaloids, etc. The estimation of the chemical diversity in rice through metabolomic approaches under various abiotic stresses is important for the improvement of new rice varieties to cope with the changing climates and possible environmental stresses in future breeding programs. Most of the previous studies done, revealed to have focused their investigations on the metabolite compositions of colored (pigmented) rice, rice bran, and bran oil which might be due to their beneficial health features like antioxidant, anti-inflammatory, and anti-hypercholesterolemic activities. Some of the metabolic features that are associated with tolerance or sensitivity could serve as markers in metabolomics-assisted breeding to improve stress tolerance in rice. However, very little is known about the comparative metabolite profiles that contribute to various abiotic stress tolerance in rice making targeted breeding strategies difficult. Increased biosynthesis, or decreased degradation, or a combination of both could contribute to the abundance of a metabolite and clarifying the relative contributions of these processes needs further attention. Accumulation of specific metabolites during stress could simply be a response to the damage caused by stress and not necessarily an adaptive mechanism that delivers tolerance. Metabolite accumulation could also result from growth inhibition as the sink strength of the particular organ is compromised during stress (Hummel et al., 2010). However, except to that of the stress specific metabolites, some novel metabolites derived from specific plant part in a tolerant genotype might play an active role as a regulator or substrate conjugate and serve some unknown function associated with alleviating the negative effects of various abiotic stresses.

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