Exploring Rice Metabolomics for Nutritional Enhancement and Stress Resilience

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Rice is a staple food crop of the world and supports the livelihood of numerous farm families providing energy requirement of billions of people and playing a pivotal role in agro-ecosystem and bio-diversity and also sustains more than half of the global population, especially in Asia. The great opportunities of rice cultivation under varied agro-ecosystem can be successfully explored through intervention of integrated soil and crop management approaches for the benefit of human nutrition and thorough insight on rice Metabolomics for enhancement of nutritional profile and stress resilience for planning futuristic agricultural research to develop highly productive and qualitative rice-based cropping system with sustainable footprints. The diversity of metabolites in rice reflects its nutritional quality, stress response and adaptation to varying environments. Metabolomics, as an emerging omics tool, has revolutionized our understanding of plant metabolism, offering insights into genotype-phenotype linkages. This chapter presents a comprehensive account of rice metabolomics, summarizing studies across nutritional profiling, abiotic and biotic stress responses, and germplasm-specific metabolic diversity. The chapter also emphasizes metabolite-based breeding strategies and the integration of genomics and metabolomics for crop improvement.

Keywords: *Rice, Metabolomics, Abiotic stress, Biotic stress, Heat map*

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Introduction

Rice (*Oryza sativa L.*) is one of the most important global food crops, particularly in Asian countries. It serves as a nutritional source in addition to the source of energy. Rice accumulates different kinds of rice-specific metabolites (Yang et al., 2014). Metabolome represents the quality and quantity of all the metabolites (<1500 Da) of a species (Kusano et al., 2007). In addition to genomics and transcriptomics, metabolomics is also useful to identify gene functions and safety assessment of genetically modified crops. Metabolomics creates an opportunity to illustrate gene functions directly related to yield, quality, nutrient content and defense mechanisms in rice (Oikawa et al., 2008). Metabolomics data could help in effective utilization of biodiversity of rice cultivars and landraces (Kusano et al., 2015; Oikawa et al., 2008). Metabolome identified 2800 QTLs for 900 metabolites and 24 candidate genes mainly accountable for the rice phenolic chemicals quantity (Gong et al., 2013). Along with commercial rice, considerable studies on metabolomics were carried out in wild rice varieties (Zaghum et al., 2022). Higher amounts of beneficial chemicals found to be accumulated in rice grains with large vacuoles revealed through metabolomics in differently shaped embryos, indicating the scope for maximum nutritional grain quality of rice (Zhao et al., 2019). Metabolomics was deployed for trait improvement (Daygon et al., 2017), biotic and abiotic stresses (Fukushima et al., 2020; Du et al., 2022; Da Costa et al., 2022; Lan et al., 2023). In general, the outer layers of the rice grain contain pigments (Juliano, 1985) and are rarely observed in the endosperm (Shao et al., 2014). Traditional rice comprises pigmented and non-pigmented rice. Most of the global population (~85%) consume non-pigmented rice. Pigmented rice consumed mostly in China, Japan, and Korea in advantage of health benefits and distinct flavor (Deng et al., 2013). Equipment like GC-TOF/MS [Comprehensive Two-Dimensional Gas Chromatography coupled with Time-of-Flight Mass Spectrometry] or 2-dimensional GC X GC-TOF/MS, LC-MS [Liquid Chromatography-Mass Spectrometry], CE-MS [Capillary Electrophoresis-Mass Spectrometry], LC-NMR [Liquid chromatography (LC) and nuclear magnetic resonance (NMR)] and Fourier transform ion cyclotron resonance (FT-ICR) MS (Oikawa et al., 2008) were used in metabolomics. Among these, GC-MS is highly popular since it is less expensive, reproducible and highly sensitive (Kusano et al., 2007). Metabolomics of three (black, red and white) germinated rice was analyzed using NMR (Pramai et al., 2018), one japonica and one indica rice germinated at various temperatures were analyzed using LC/MS (Yang et al., 2019) and twelve popular cultivars using GC/MS (Ranjitha et al., 2019). Among the sustainable development goals, SDG2 targeted zero hunger by achieving food security and improved nutrition. Rebuilding the immune system through a nutrition rich diet is the need of the hour to combat health catastrophes. Lately, it is realized that traditional rice varieties constitute a valuable gene pool for features that may be utilized in developing nutrient rich cultivars. Considering the volume of published literature, an attempt is made to bring all the publications on rice metabolomics in place and presented in this chapter for the benefit of readers and scientific community and other associated stakeholders interested in rice metabolomics.

Abiotic stress: Several studies have explored the metabolomic response of rice cultivars under abiotic stress conditions. Drought (Ma et al., 2016) Salinity (Gayen et al., 2019; Gupta & De, 2017), heat (Mishra et al., 2024), Nutrient (Zhao et al., 2018).

Heat stress: Significant variations in the concentrations of 64 metabolites in heat maps indicate variable response in twelve (BR2655, Thanu, Tunga, BPT5204, etc.) cultivars using methanol/water (4:1) with GC-MS in full scan as well as selected reaction mode (SRM) analyses in heat stress (Ranjitha et al., 2019).

Cadmium (Cd) stress: A total of 103, 51 and 50 metabolites showed significant differences in the root, stem and leaf tissues, respectively of *TanLiangYou215* (TLY215) was studied using methanol–water (80% *v*/*v*) solution and HPLC-QTOF/MS under full scan mode. Upregulation of amino acids (alanine, aspartate, glutamate, and proline) and flavonoids (strong antioxidants) pathways prevented oxidative damage in roots. However, stem and leaves, significant down regulation of glycerophospholipid metabolism helped the adaptability of *TLY215* to cadmium stress (Lan et al., 2023).

Giant embryo rice: Giant embryo rice (*O. sativa* L. 89 *japonica* cv. *Dapeimi*) showed differential accumulation of 233 metabolites (amino acids, sugars, organic acids, and nucleotides) to tolerate abiotic stress conditions using methanol–water (80% *v*/*v*) and UHPLC/MS/MS GC/MS under full-scan mode (Zhao et al., 2018).

Nutritional trait variation under controlled conditions: Varying nutritional metabolites (protein, fiber, phenols and carotenoids) were noted in grey and Zag rice lines in environmental stress (Ashraf et al., 2017).

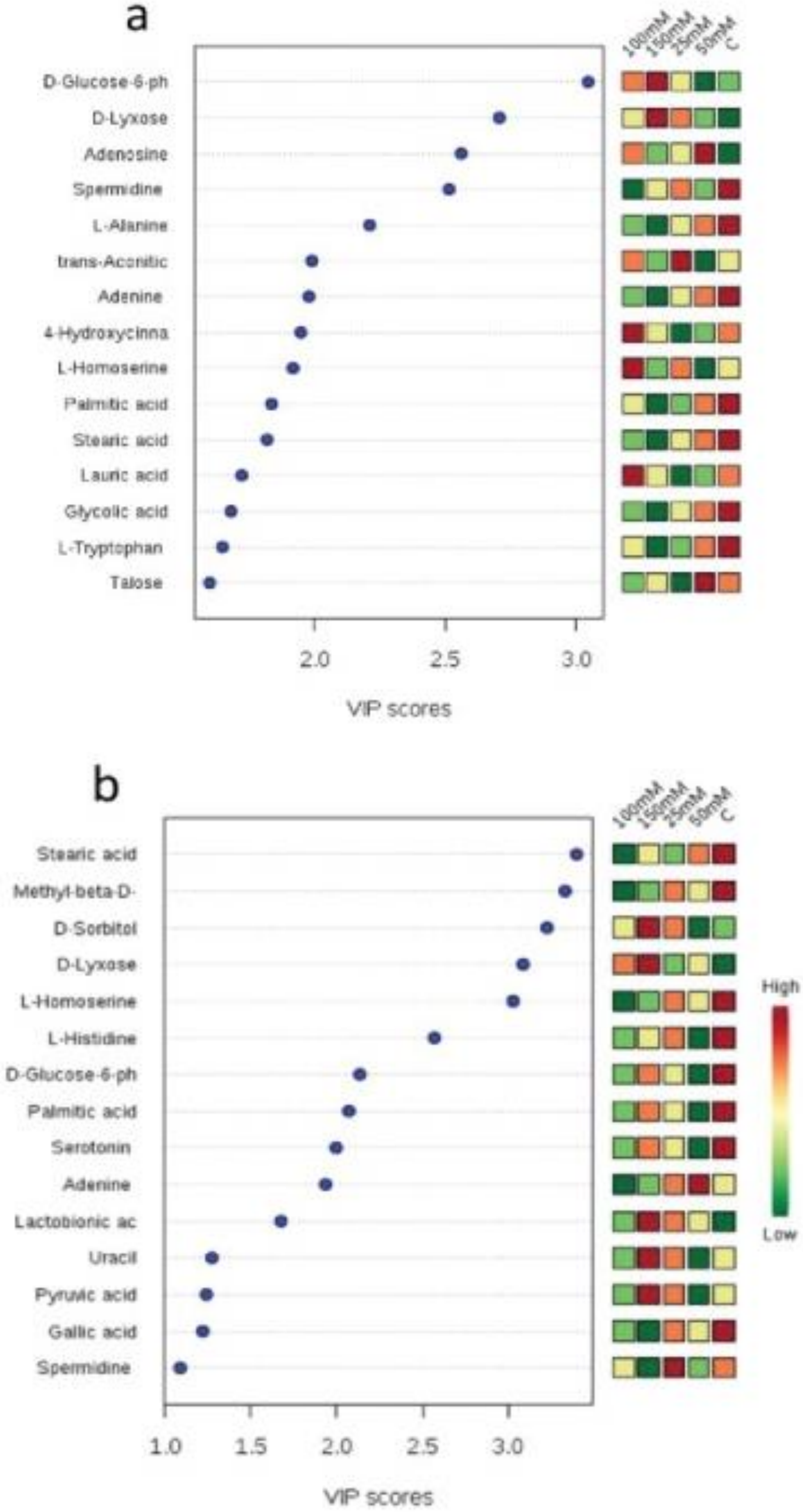
Low nitrogen stress: A backcross introgression line (G9) showed elevated levels of amino acids, organic acids, and sugars than its recurrent parent (SH527), particularly in pathways related to glycolysis, TCA cycle, and nitrogen assimilation and these metabolic changes help to circumvent the stress (Zhao et al., 2018).

Salinity stress: In a salinity stress study, two salt-sensitive and two salt-tolerant indica rice varieties were evaluated using GC-MS. The tolerant genotypes (Bhutnath, Nonabokra) exhibited increased accumulation of signaling molecules (serotonin and gentisic acid), ferulic acid and vanillic acid (Figure 1). In contrast, the sensitive varieties (Sujala, MTU 7029) accumulated 4-hydroxycinnamic acid and 4-hydroxybenzoic acid (Gupta & De, 2017).

Water stress: Accumulation of osmolytes, glycolytic intermediates, TCA cycle metabolites, and auxin conjugates were observed under submergence in deep water rice (C9285, NIL-12) in comparison with non-deep-water rice (T65) using multi-platform metabolomics (GC-MS and CE-MS). This metabolic adaptation is useful to circumvent deep water stress (Fukushima et al., 2020).

Light stress: Upregulation of chloroplast P450 monooxygenase (CYP97A4) that enhances provitamin A content (lutein) biosynthesis and plays a critical role in protecting against light-induced oxidative stress in *Nipponbare (japonica)* rice (Lv et al., 2012).

Drought tolerance: Compared to IR64, N22 showed higher activity of HMG-CoA reductase (a physiological adaptation) leading to higher accumulation of phytosterols (campesterol, stigmasterol and β-sitosterol) that stabilized plant cell membranes under drought stress (Kumar et al., 2015). This highlights the potential use of phytosterol accumulation as a biochemical marker for drought resilience in rice breeding programs. Similarly, upregulation of 4-hydroxycinnamic acid and ferulic acid in the tolerant genotype (IAC1246) than IRAT109 (susceptible) (Ma et al., 2016). Together, these investigations affirm that rice germplasm exhibits extensive natural diversity in metabolite profiles, shaped by pigmentation, subspecies lineage, tissue specificity, and stress responsiveness. This metabolite diversity holds immense potential for developing nutritionally rich, stress-resilient, and functionally enhanced rice varieties through breeding and metabolic engineering. Varietal specific metabolites were used as biomarkers for abiotic stress and nutrition starvation (Kusano et al., 2015). Metabolomics was extensively used to monitor and assess the cellular metabolism and quality of rice (Okazaki & Saito, 2016). Two rice varieties having varying nitrogen tolerance were cultivated at different nitrogen levels and were subjected to metabolomics analysis (Zhao et al., 2018). Metabolomics has been carried out in Indica as well as Japonica rice (Hu et al., 2014), traditional rice cultivars (Udhaya et al., 2023), landraces and cultivated rice (Zhang et al., 2022).



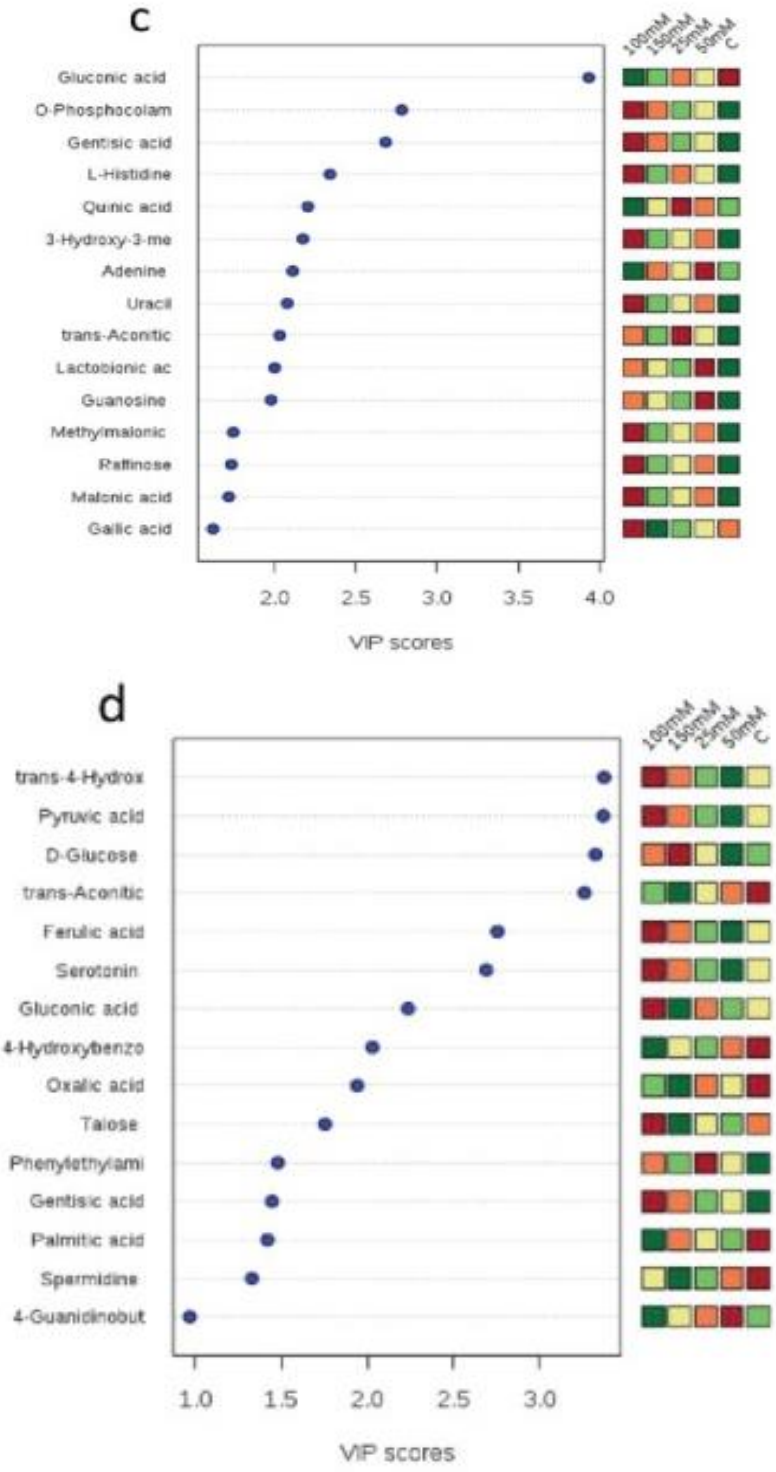


Figure 1. Variation in levels of *metabolites in susceptible ((a) Sujala; (b) MTU 7029) and tolerant ((c) Bhutnath; (d) Nonabokra) salinity stress* (Adopted from Gupta and De, 2017). VIP is variable importance in projection used in the partial least square discriminant analysis (PLS-DA) model as a quantitative estimation of the discriminatory power of each metabolite. VIP scores of ≥1 were considered important.

Bioactive metabolites in pigmented and traditional rice

Pigments are naturally occurring compounds. They belong to the family of flavonoids and are glycosylated (aglycone), methylated or acylated forms of anthocyanidin. Red / purple / black colour in pigmented brown rice is due to various combinations of cyanidin-3-O-glucoside and catechin (Goufo & Trindade, 2014). They also contain genetically dependent levels of carotenoids (Ashraf et al., 2017). Pigmented rice varieties have gained attention in recent times, particularly red and black rice, due to their bioactive compounds that boost immunity. Rice with darker shades possesses qualities that go beyond those found in light-colored varieties (Subramanian et al., 2024). Multiple studies on traditional and modern rice cultivars using high-throughput analytical techniques such as UPLC-MS, GC/MS, HPLC, and UHPLC-QTOF-MS2 to understand the metabolomics. Varied levels of gama amino butyric acid (GABA) was noticed in 68 using GC-TOF/MS suggesting the need for selecting health-beneficial varieties and highlights new trait-based breeding strategies (Kusano et al., 2007). Black, red and dark purple rice varieties are rich in anthocyanins, which were identified using HPLC. Key compounds include cyanidin 3-glucoside, cyanidin 3-galactoside, cyanidin 3-rutinoside, cyanidin 3, 5-diglucoside, malvidin 3-galactoside, peonidin 3-glucoside, and pelargonidin 3,5-diglucoside (Deng et al., 2013*)*. Of the 121 metabolites, differences in nutrient storage, oxidative stress tolerance, and adaptation strategies between subspecies (51 japonica and 49 indica cultivars) were observed (Hu et al., 2014). Of the 58 varieties, black rice had the highest contents of vitamin E (α-, β-tocopherols, and α-tocotrienol) over others including red rice which indicates that both grain color and genotype influence vitamin E composition (Shammugasamy et al., 2015). Tissue-specific analysis of white (9311), red (SB7), and black (Yunanheixiannuo) rice grains revealed that bran and embryo fractions had significantly higher phenolic compounds and antioxidant capacity than endosperm which indicates that both genotypic effects and tissue localization are better than rice bran in nutrient composition (Shao et al., 2014). In Habataki, high-resolution LC–QTOF-MS/MS and NMR analysis identified 36 flavonoid (five novel) and eight rare flavonolignan isomers suggesting cultivar-specific accumulation of phenolics (Yang et al., 2014). In aromatic rice 2-acetyl-1-pyrroline (2AP) the key compound responsible for aroma was also noticed (Daygon et al., 2017). Metabolic engineering was used successfully to biosynthesize betanin (red pigment) in the endosperm of Zhonghua11 (japonica rice) by transgenic expression of meloS, BvDODA1S, and BvCYP76AD1S genes without reduction in grain yield or plant growth (Tian et al., 2020). Compared with white (Baegilmi) and brown (Hyunmi) rice, black rice (Chalheugmi) contains ferulic acid, caffeic acid, epicatechin, quercetin, etc. of phenylpropanoids and flavonoids groups (Tyagi et al., 2022). In LC-MS analysis, higher anthocyanin accumulation in 6 landraces was observed over 4 cultivars because of higher expression of anthocyanin glycosylation-related genes pointing to genotype-specific regulation of secondary metabolite pathways (Zhang et al., 2022). Extrusion puffing of Zhongzi 4, a black/purple rice variety (Ma et al., 2023) significantly enhanced various nutrients (flavonoids, minerals, and proteins) and improved the diversity of volatile compounds leading to the overall nutritional and sensory profile. This suggests that food processing techniques can enhance the bioactive profile of pigmented rice making them more valuable as functional foods. Metabolomic profiling of five traditional cultivars from Tamil Nadu (Chinnar, Chithiraikar, Karunguruvai, Kichili samba and Thooyamalli) revealed the presence of 149 metabolites spanning 34 chemical classes- alkaloids, dihydrofurans, fatty acyls, hydroxy acids, organooxygen compounds, prenol lipids, saturated hydrocarbons and steroids. This emphasizes the untapped nutritional and medicinal potential of traditional landraces and highlights their relevance for crop improvement, agro-biodiversity conservation and policy initiatives to support their cultivation (Udhaya et al., 2023). Among the 139 metabolites (anthocyanins, flavonols, tannins, and other polyphenolics) identified through UPLC in four colored Kam Sweet Rice green (Lvhe), purple (Heihe), red (Honghe) and white (Goujingao), highest anthocyanin, flavonoid and vitamin B1 and E contents were found in red and purple rice (Liang et al., 2023). Sakuranetin (flavonoid) content was enhanced in the endosperm of transgenic biofortified rice Zhonghua11 by overexpressing a naringenin 7-O-methyltransferase (NOMT) gene (Figure 2) under the OsGluD-1 promoter (Zhao et al., 2024). GC-MS/MS revealed that a red rice (Kullakar) had lower levels of stearic acid, palmitic acid, and ribavirin (Figure 3) than white rice (Milagu Samba) suggesting that pericarp pigmentation influences metabolic composition (Subramanian et al., 2024). Germination significantly increased the nutritional profile of pigmented rice sprouts (PRS) free phenolic compounds, essential secondary and micronutrients (Ca, Na, Fe, Zn) Vitamins (riboflavin and biotin), and bioactive compounds (γ-aminobutyric acid (GABA), dipeptides, and flavonoids) and simultaneously reduced the anti-nutritional compounds (proanthocyanidins). Further, the random forest classification algorithm categorized the 600 metabolites of PRS into four distinct clusters (Tiozon et al., 2023). Besides, flavonoids (tricin, luteolin, apigenin, quercetin, isorhamnetin, kaempferol, and myricetin) exhibit diverse bioactivities (antioxidant, anti-inflammatory, and anti-cancer properties) through mechanisms like radical scavenging, cell signaling modulation, membrane interactions and inhibition of angiogenesis and cell proliferation (Singh et al., 2017; Deng et al., 2013) and are useful against diseases like diabetes, cancer and cardiovascular ailments (Tyagi et al., 2022). This underscores the therapeutic potential of flavonoid-rich rice varieties and their value in biofortification strategies. Metabolome profiling using integrated platforms (GC–MS, LC–MS, CE–MS) cataloged a broad range of primary and secondary metabolites in diverse rice cultivars (Kusano et al., 2015), highlighting the metabolic complexity of the species. The genetic background, pigmentation and metabolic engineering significantly influence the rice metabolome leading to precision breeding, functional grain development and stress-resilient cultivar selection.

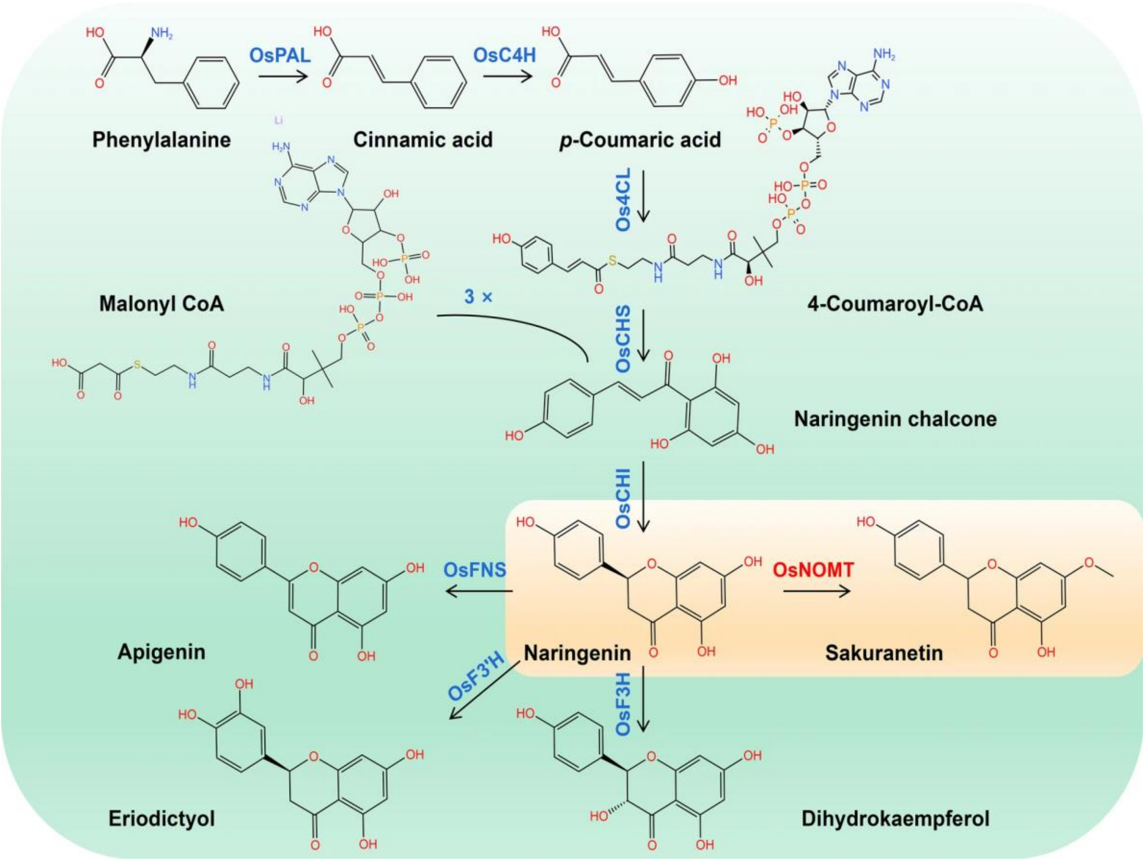


Figure 2. The pathways of sakuranetin biosynthesis in rice. Metabolites are indicated in black and different enzymes are indicated in color (Adopted from Zhao et al., 2024).

Biotic stress

Rice metabolomics play a key role for mitigating biotic stresses including pest and diseases. Different metabolites as biomarkers for resistance against Gall midge of rice for identification of susceptible and resistant cultivars have been studied by the researchers for combating the attack by the menacing pest in rice. Among the biotic stresses investigated, the bioactive compounds responsible for disease resistance in rice for Bacterial leaf blight, mode of action etc was studied by (Sana et al., 2010), and the rice metabolomics and their mechanism of resistance to sheath blight of rice caused by *Rhizoctonia solani* was documented by (Suharti et al., 2016).

Gall midge infestation (biotype 1)

Differential accumulation of organic acids, fatty acids, sugars and amino acids were noticed in three rice cultivars (TN1, Kavya and RP2068) infested by *Orseolia oryzae* (gall midge) using GC-MS indicates these

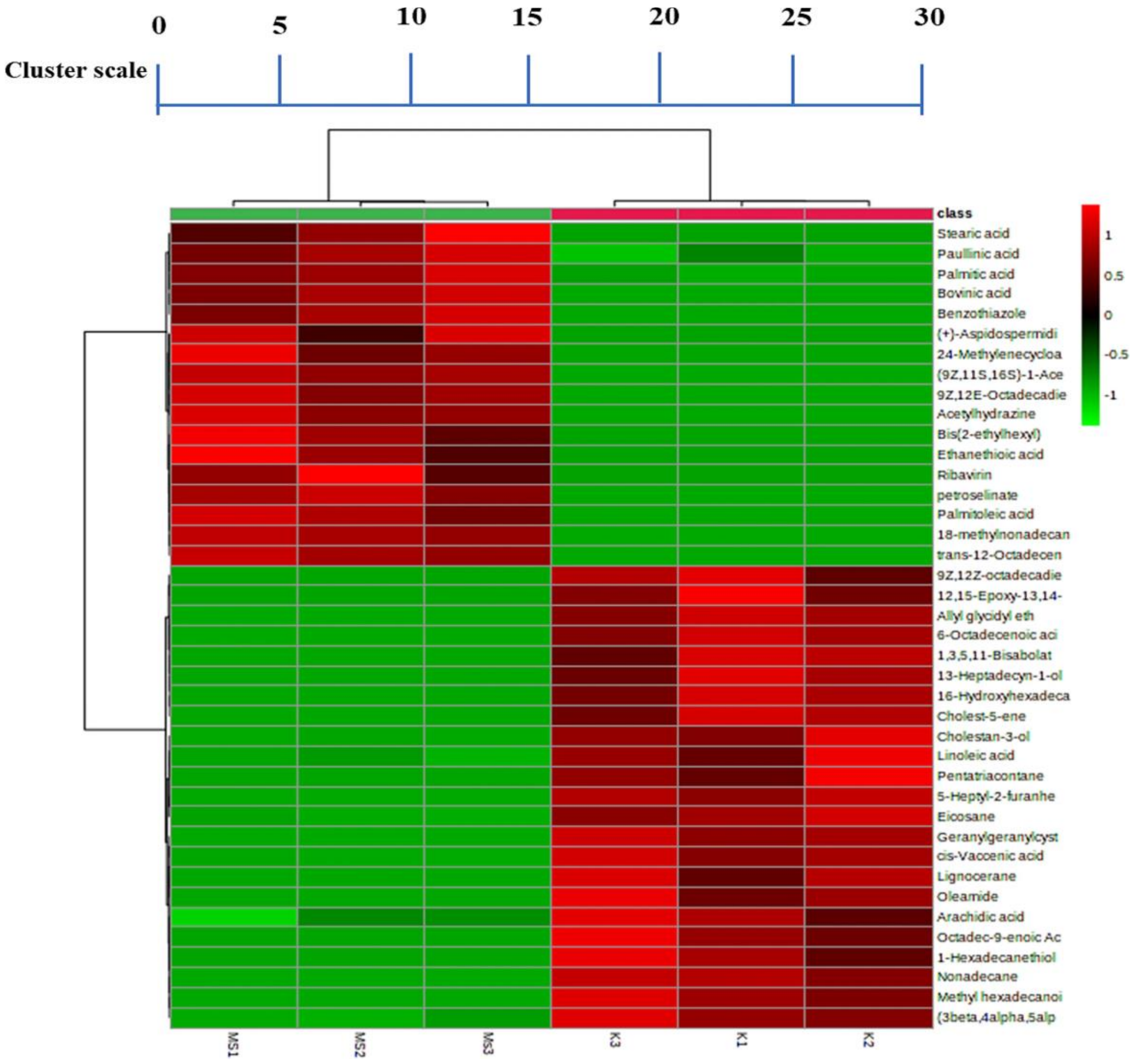


Figure 3. Heat map of differential metabolites between the grains of Kullakar (K1, k2 & k3) and Milagu Samba (MS1, MS2 & MS3). Cells in red (higher) and green (lower) indicate relative abundance of the metabolites in the rice samples (Adopted from Subramanian et al., 2024).

metabolites as biomarkers for resistance against this pest and to identify susceptible and tolerant genotypes (Agarrwal et al., 2014).

Sheath blight

Presence of higher levels of jasmonic acid, mucic acid, and glyceric acid and differential induction of ADP and phenylpropanoids in resistant (32R) over susceptible (29S) rice lines indicates the importance of these metabolites in host defense and stress respectively against *Rhizoctonia solani* (sheath blight pathogen) (Suharti et al., 2016).

Bacterial leaf blight (BLB)

Metabolomic profiling using GC–TOF-MS and LC–TOF-MS under varying conditions revealed (Figure 4) changes in organic acids (citric acid, succinate, glyoxylate) and sugars (glucose, fructose) in TP309 (susceptible to BLB pathogen- *Xanthomonas oryzae pv. oryzae* (Xoo)) rice leaves using pre-cooled (−20°C) 2:3:3 v/v/v solvent mixture of water/acetonitrile/isopropanol under full scan mode (Sana et al., 2010).

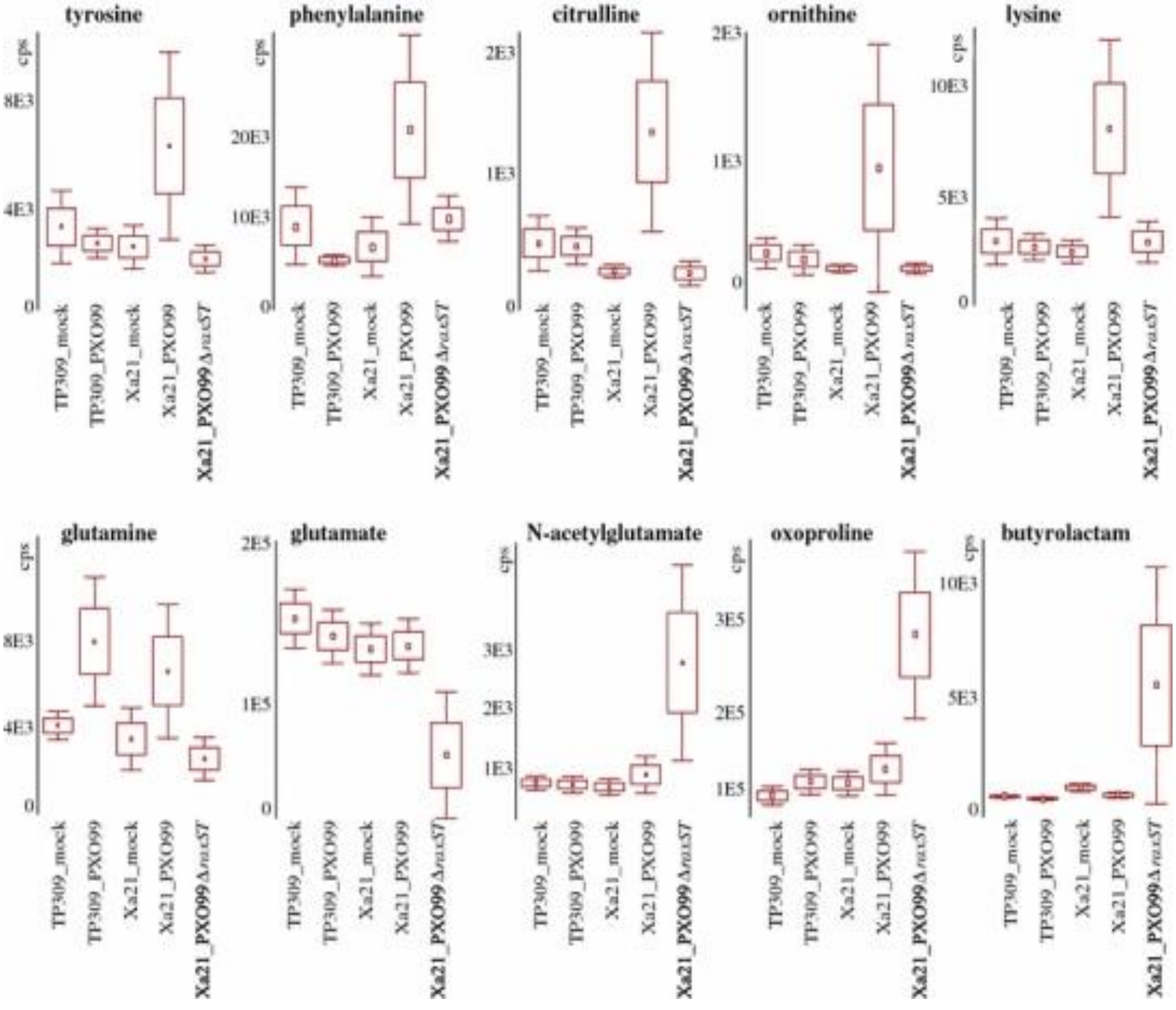


Figure 4. Differences in rice leaves infected with PXO99 (resistant) or PXO99_ΔraxST (susceptible) TP309 plants (Adopted from Sana et al., 2010).

Way forward

Ex-situ gene banks are invaluable repositories of genetic diversity with extensive collections of landraces, wild relatives and traditional cultivars (Sreenivaasulu et al., 2023). Significant advances in genome sequencing, metabolomics, lipidomics, and ionomics are accelerating the characterization of gene bank accessions. These tools are useful to dissect complex nutritional traits, discovery of metabolite-linked genetic loci and development of molecular markers for trait specific breeding. The priority traits for nutritional improvement are 1) Proteins and essential amino acids, 2) Polyunsaturated fatty acids (PUFAs), 3) Resistant starch and dietary fiber, 4) Vitamins (A, B-complex, E) and 5) Carotenoids and other phytonutrients. Enhancing these traits in staple crops through breeding and biotechnological strategies is central for promoting health in resource-limited populations. Besides, the challenges and gaps include 1) post-harvest refining and processing often deplete essential nutrients, 2) Data integration and accessibility across gene banks and platforms remain limited and 3) Certain micronutrients and metabolites remain under-investigated due to analytical or funding limitations. Improving phenotyping pipelines, data infrastructure, and interdisciplinary collaboration is critical to overcoming these barriers. Such integrative efforts can inform and enrich breeders, policymakers, and public health professionals to design, develop and promote staple crops with superior health-promoting attributes, contributing to both human well-being for nutritional security, development of immunity, resistance to diseases and sustainable agricultural development.

Conclusion

The metabolomics system is a robust tool for studying stress analysis. Traditional and pigmented rice germplasm exhibit a wide range of bioactive metabolite profiles, reflecting their adaptation to diverse environments and crop ecology and also potential for health benefits. Collectively, these studies underscore the metabolomics dynamics and richness of rice germplasm and its relevance in tailoring breeding strategies for improved nutritional quality and stress resilience, that play pivotal role for global nutritional security under the backdrop of climate resilient agricultural system. The integration of metabolite profiling with genomics opens promising avenues and a new era for metabolic trait-based selection, genome editing and precision breeding. These metabolite signatures may serve as biomarkers for salt tolerance. The value of germinated pigmented rice as a functional grain capable of addressing hidden hunger and non-communicable diseases, micro-nutrient malnutrition prevalent in rice-based diets. The study presents a robust integrative framework that leverages metabolomics and genomics to inform biofortification breeding strategies, thus supporting the development of rice varieties with enhanced health benefits. Metabolomics has now emerged as a powerful tool to characterize the biochemical signatures of diverse rice germplasm, elucidating how metabolite accumulation varies with genotype, tissue type, and stress condition, as well as through targeted bioengineering.

Conflict of interest: Authors declare no conflict of interest.

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