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METABOLOMICS INSIGHTS INTO RICE STRESS RESPONSES UNDER CHANGING CLIMATE

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ABSTRACT

More than half a world relies on rice as the source of calories; however, it is facing mounting threats of heat, drought, flooding, salinity, cold and growing levels of atmospheric CO2 the dangers often do not happen in isolation but happen together. Understanding of molecular response of rice to multiple environmental stresses has become a major priority modern crop science. The review brings together existing metabolomics studies on rice responses to stress, the objective of which is to unravel shared metabolic responses to six major abiotic stresses, to establish the impact of soil typology on these responses, and to determine the key knowledge gaps that have a direct impact on breeding programmes. Available published metabolomic studies utilizing either gas chromatography/ mass spectrometry (GC-MS), liquid chromatography/ mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) were analyzed and related to the soil-science literature with the aim of placing metabolic findings in real agronomic situations. It used evidence based on both the controlled experiments and free-air CO2 enrichment (FACE) field trials. In all stress conditions, proline, γ -aminobutyric acid (GABA), and raffinose -family oligosaccharides became conserved protective compounds, whereas a general inhibition of tricarboxylic -acid (TCA) intermediates indicated metabolic stress and resultant yield loss. Heat stress alters the biosynthesis of starch, resulting in chalky grains; drought stimulates the accumulation of proline and trehalose, which is regulated by soil texture; flooding shifts the carbon flux to fermentative materials; salinity induces the accumulation of glycine-betaine; cold changes lipid composition of the membrane; and the increased CO2 selectively downregulates the concentration of grain protein, zinc, and iron - a phenomenon enhanced by nitrogen deficiency in the soils. Many of the metabolites can currently meet the metabolite-assisted breeding selection criteria. Metabolomics is no longer just another descriptive narrative; it has developed into quantifiable, inheritable biomarkers with an actual potential to guide the creation of stress-resistant rice crops modified to cope with a climate that is growing more erratic.

KEYWORDS: rice metabolomics; abiotic stress; proline; GABA; raffinose; TCA cycle ; climate change; stress tolerance; biomarkers; breeding

1. INTRODUCTION

The relationship between rice farmers and the climate, shifted over the past two decades, is among the most fundamental changes globally. The climate altered the seasonal rhythms due to its impact on the way moisture appears on agricultural surfaces. The situation involves a transition of predictable cycles, and the erratic rainfall of the monsoon reflects the environment's idea of the connection between nature and humanity. The complex and vulnerable rice cultivation contains various elements of growth, such as rainfall, temperature, and soil, as well as the principles, for instance, carbon flow and biochemical networks. The climate used numerous elements of change in rice production, for instance, erratic patterns to represent instability and vulnerability (22, 36, 83). The environment contains several repeating disruptions from the flash floods and the saltwater in the deltas. Environmental shifts used erratic patterns which created a disrupted flow and slightly lighter grains; however, the harvest contains slightly lower quantities (7). Numerous disruptions give the grain weight outlining areas where the leaf turns to grain and photosynthesis changes to yield.

The science of metabolomics allows one to monitor such perturbations on a real-time basis at a molecular level. In plant tissues, metabolomics determines hundreds of small molecules, including lipids, organic acids, amino acids and secondary metabolites at the point of stress rather than waiting until harvest time, or when measurable stress signs appear, such as leaf rolling or bleached florets, occur (12, 49). It is the metabolome which combines information about gene activity, enzyme activity, and the environmental conditions into a consistent picture that becomes a reflection of the functional state of the plant, not its functional potential

Tools for analysis now let us do this type of profiling widely. Key components of central metabolism such as amino acids, sugars, organic acids are easily identified with gas chromatography –mass spectrometry (GC-MS) reliable results and wide range (2, 25). Beyond that signals associated with cell activity, lipids and secondary molecules are detected by liquid

chromatography mass spectrometry (LC-MS) . Meanwhile, nuclear magnetic resonance (NMR) gives data without harming samples, adding depth where others might miss. From all these methods combined, we've built a solid body of work showing how rice changes its chemistry under heat, lack of water, too much water, salt, cold, even higher CO_2 levels. Despite this progress, the literature has notable gaps. The majority of published metabolomics studies use a small number of model genotypes to study a single stress at a time under carefully regulated growth-chamber conditions. In actuality, rice grows in soils that differ greatly in terms of nutrient supply and water retention, is subject to numerous stresses at once or quickly after, and comes in thousands of genetically unique genotypes. This review tries to bridge that gap by synthesizing what controlled metabolomics studies have found while pointing clearly to where field-relevant knowledge still falls short. The organization adheres to the reasons behind the stresses; section 2 covers each major stress and how the soil context shapes the response; section 3 covers the central metabolic pathways; section 4 stress interactions are covered; single stress experimental evidence in section 5; The information gaps and breeding implication are mapped in section 6; section 7 concludes

2. Abiotic Stresses and Their Metabolic Consequences in Rice

All forms of abiotic stresses subjected to rice have a unique biochemical profile in the tissues of the plant. Although some of the metabolite e.g proline and gamma-aminobutyric acid (GABA) can be increased in response to a large range of stressful environments, features of these signatures can be distinctly different with unique aspects reflecting the mechanism of damage. It is also noteworthy that responses to metabolic stress do not occur in a vacuum where the soil environment, water-holding capacity, organic matter content, pH and mineral availability all have an effect in moderating the repertoire of metabolites available to the plant when it is exposed to stress, and, thus, having an effect on the intensity of the response to be taken before nitrogen, carbon, or energy resources become depleted. The following subsections integrate soil context as an inseparable part of the metabolic narrative instead of considering it as a background issue.

2.1 Heat Stress

An exposure of two to three days to temperature issues of more than 35 °C during anthesis or grain filling causes a quantifiable loss of pollen viability, spikelet fertility, and starch deposition. The harm to crops in such environments comes about due to the inability of reproductive processes and not due to death of the plants (3, 24, 55). Heat stress has been a widely studied topic in terms of its metabolomic footprint in rice.

Even as the TCA-cycle intermediates malate, citrate and succinate continue to rapidly decrease due to the onset of the mitochondrial malfunction, continue to rapidly decrease due to the onset of mitochondrial malfunction, a dramatic increase in proline, GABA, galactinol and raffinose is observed in the rice tissues (6, 87). There is also the decrease in the production of starch, as AGPase and GBSS gradually decreases at 33°C. When heat occurs, the phenylpropanoid pathway brings nucleic acids and membranes under control by pumping out flavonoids, hydroxycinnamic acids and rushing putrescine, spermidine, and spermine in the event of heat stress (46, 56, 76).

These metabolic reactions are highly dependent on soil type. In lowland rice systems with paddy soils that are based on alluvial clay with a high level of nitrogen, proline biosynthesis is less inhibited by the availability of nitrogen due to the presence of adequate substrate in the glutamine synthetase (GS)/glutamate synthase (GOGAT) cycle (10, 53). As a result, the metabolic response would last longer before the catabolic breakdown of protein reserves. Conversely, soils in lowlands, particularly those found on uplands, are more often subject to both incipient drought and nitrogen deficiency, which limits the metabolic resources at the start of the synthesis of protective compounds (26, 34). Varied in their levels of tolerance, tolerant cultivars are always in high soluble sugar reserves and more stable amino-acid pools under heat stress than susceptible lines (11, 66, 81, 86).

2.2 Drought Stress

When roots sense dry soil, leaves react fast - long before they droop. As water pressure inside cells dips, tiny pores on the surface seal shut, triggered by a surge in ABA hormone levels. With those openings closed, fresh air stops flowing in, so energy production from sunlight loses steam. Less sugar moves down to young seeds forming in the ear. Fuel sources feeding core metabolism dwindle at the same time demand rises elsewhere. Survival shifts priority cells redirect materials into holding onto moisture instead of adding new tissue. Growth pauses without warning. When rice faces dry conditions, proline builds up more than almost any other substance seen during plant stress often five to twelve times normal amounts, occasionally even more fulfilling several roles at once by helping cells retain water, reducing oxidative damage, and protecting proteins from breaking down due to drying (28, 72). Under similar stress conditions, concentrations of trehalose and its precursor trehalose-6-phosphate increase, not only to equilibrate turgor but also to convey signals that modulate the distribution of assimilates between foliar tissues and developing seeds (8). Plants that catabolise proteins experience accumulation of the branched chain amino acids valine, leucine, and isoleucine as substrates of cellular respiration and the depletion of asparagine and glutamine due to the re-direction of nitrogen reserve to urgent biosynthetic needs instead of storage (50, 62)

Different soil types shapes how fast plants feel dry spells. Clay-heavy floodplain earth holds moisture longer compared to loose sandy ground above, which means crops there manage without rain for longer - metabolism shifts happen slower, hit softer (9, 18). When soil is coarse, thirst strikes quick after rains stop, pushing proline way up while slicing through TCA cycle parts faster. Laterite or sour-soils bring extra trouble during drought: toxic aluminium joins in, forcing roots to leak acids like malate and citrate as protection an escape move that drains stored carbon even more (38, 78).

2.3 Flooding and Submergence Stress

The entirely different metabolic stress caused by submergence is not cellular in nature: the rice tissues must deal with the problem of oxygen deficiency due to the limited exchange of gases between submerged tissues and the atmosphere. Root and shoot tissues switch to anaerobic fermentation and inhibit pyruvate oxidation, diverting it to the synthesis of ethanol by pyruvate decarboxylase and alcohol dehydrogenase and of alanine by alanine aminotransferase, within hours after being completely submerged. GABA, ethanol, lactate and alanine in submerged tissues build up to form a metabolic fingerprint of early and repeatable anaerobic stress. Even though these fermentative pathways maintain energy generation in the anaerobic environment, their energy productivity per mole of glucose is much less than aerobic respiration causing rapid dissipation of soluble carbohydrate stores. Assuming that the submergence continues, the stores can be taken away by the time that the water will be re-aerated and not enough carbon substrate will be available to enable the regeneration of the growth after floods will have subsided.

In this case, GABA shunt becomes dominant and the TCA cycle is bypassed in such a way that the flow of carbon does not cease, even in case the oxygen-linked reactions slow down (57, 65). Alanine gets deposited during oxygen deficiency and instead of being a source of damage, it is a safe storage of both carbon and nitrogen. Due to the presence of Sub1A gene, some rice varieties will not ferment during a period spending underwater where they will store sugar rather than fermenting; their dormant metabolism allows them to survive complete flooding by almost two weeks before they begin regenerating (14, 23, 48, 64). After the water has gone away, air has returned to the tissues, the mitochondrial activity is restored followed by the generation of reactive oxygen species after which flavonoids and phenolic acids are amassed, thus preventing oxidative damages to membranes (46, 79).

The type of soil alleviates the severity of metabolic stress caused by submergence at its natural level due to its effect on the water retention and chemical composition. In low hydraulic conductivity clay soils detailed hypoxia phases of the root zone persist even after surface water recedes, extending the pathway of hypoxia metabolic programme, and bordering aerobic respiration. The soils of peat are a special problem: during anaerobic decomposition of organic matter, phenolic acids, volatile fatty acids, and other phytotoxic substances are produced, which are discharged into the flood water and diffused into the root zone. These compounds have the potential to interact with secondary metabolite biosynthetic pathways in rice and there may be the induction of defence responses which may add extra energetic loads on tissues that are already functioning within harsh carbon conditions. The overall consequence of this is that submergence tolerance depends not purely on genotype, but significantly changed by the physical and chemical characteristics of the soil environment.

2.4 Salinity Stress

Salts cause two components of stress, an osmotic perturbation caused by high concentrations of external solutes that decrease cellular water potential, and an ionic perturbation caused by sodium and chloride ion influx into plant tissues that disrupts enzymatic functions and disrupts the uptake of essential nutrients (13, 16). Rice is moderately sensitive to salt and this is especially sensitive during the reproductive phase where ionic build-up is prone to occur in floral structures. The metabolic salinity response, to some degree, reflects the proline and γ -aminobutyric acid accumulation in response to drought and flood, but also has some unique characteristics. The osmolytes, including glycine betaine, myo-inositol, and trehalose, accumulation is increased as a special response to ionic stress (47, 60). After Na^+ influx, raising levels of GABA in the roots are the result in large part of the cytosolic acidification of the root, which activates glutamate decarboxylase (GAD) via calcium and pH sensing systems (32, 57). Salinity increases the production of secondary metabolites, especially anthocyanins, flavonoids, and phenolic acids, and at alkaline saline soils, oxidative stress is promoted by iron deficiency (52, 84).

Under saline conditions, salt-tolerant crops like Pokkali and FL478 have been metabolomically determined to accumulate glycine betaine and inositol faster, and exhibit more stable pools of glutamine, which points to a more efficient nitrogen economy (21, 40, 67, 92). The salinity reactions are highly dependent on the soil properties. Clay-alluvial soils can reduce sodium influx through cation exchange processes, which slow the speed of ionic uptake in root tissues and provide the plant with sufficient time to activate exclusion pathways before Na^+ concentrations attain phytotoxic concentrations (10, 13). On the other hand, low cation-exchange capacity sandy soils allow free movement of sodium to the periphery of the roots, increasing the rate of the ionic component of salinity stress, enhancing proline buildup, and placing a less enduring phytotoxic strain. Alkaline saline soils also restrict the supply of phosphate and iron, which results in synergistic nutritional and osmotic stress which significantly changes secondary metabolite profiles (58, 90).

2.5 Cold Stress

The cold stress has an alternate mechanistic pathway to the above mentioned stresses. Enzymatic reactions, catalysts, membrane phospholipids, switch to a gel-phase and disrupt protein activity, photosynthetic electron transport efficiency decreases, resulting in the production of reactive oxygen species (ROS) at low temperatures (below 15 °C) (45, 74). Due to the cold exposure, the metabolic route is typified by changes in the membrane lipid composition and the production and accumulation of certain cryoprotective metabolites. The most prominent change is raffinose family of oligosaccharides, including raffinose, galactinol, and stachyose, which increase under the condition of freezing in rice and act as cryoprotectants, as they associate with bilayers of membranes to stop their transition to the gel form (59, 89). Other cryoprotectants, including proline and sucrose are amassed as well. Proline is a two-factor compound that doubles up to be an osmolyte and a reactive oxygen species scavenger during chilling. Falling levels of glutamate and aspartate suggest the GS/GOGAT system shuts down when it gets cold, blocking nitrogen movement. (69, 91).

The remodelling of the membrane lipids to improve their fluidity subsequently brings about the rise in polyunsaturated fatty acid concentrations. (19, 80). Together with that, there is an increment in concentrations of γ -tocopherol and carotenoids, which neutralize photo-oxidative damage due to excessive chloroplast excitation energy (46, 88). Cold stress dynamics are strongly dependent on the thermal properties of the soil. Soils with low drainage or water-saturation have high thermal inertia and hence the temperature change is slower compared to well-drained soils, which provide the rice roots with a slightly longer period to develop metabolic defenses before the cold signal reaches the shoot (94). Conversely, at high-altitude rice locations with thin and coarse soil types, the ambient temperature of the atmosphere is closely imitated and this triggers a quick occurrence of cold stress which requires a faster and stronger metabolic reaction. The cold tolerance is also further regulated by soil phosphorus status; the adequate phosphorus supports membrane lipid remodeling during cold acclimation, and phosphorus deficiency increases cold sensitivity (69, 88).

2.6 Elevated Atmospheric CO_2

It may seem that the presence of high CO2 promotes growth of rice. Currently, the air has about 420ppm of CO2 but it can be increased to 550-700ppm by 2100, thus increasing the photosynthetic capacity of the plant to fix CO2, but also minimizing the losses of photosynthetic energy to the atmosphere caused by photorespiration, which will result in more sugar production and accumulation of energy within rice cells (1). These developments are seeming beneficial at first. In case where carbon flux through the plant is high however, acquisition of nitrogen does not grow in the same proportion. The nitrogen-binding enzymes, especially GS/GOGAT, do not accelerate when CO2 is high hence inhibiting the synthesis of amino-acids despite the accumulation of carbohydrates. As a result, grains are nutritionally unbalanced: they are enriched with carbon and lack nitrogen-based protein constituents, which, in turn, changes the nutritional value in a measurable way (44, 82).

Rice plants under high CO2 conditions are characterized by an increase in the concentration of sucrose, starch, proline, and raffinose and a decrease in the concentration of glutamine, asparagine, and methionine, which is more indicative of carbon allocation changes than changes in water status (37, 93). Even though the accumulation of proline is often linked with drought stress, in this regard it serves as a buffer of surplus carbon in the environment when protein synthesis is limited (35). Free-air CO2 enrichment (FACE) field experiments in China, Japan, and Australia have demonstrated that rice grains have lower levels of protein, zinc, iron and B 2 vitamins, where the losses are so significant as to be of concern to nutrition experts. Protein content, zinc, and iron content were reduced by 5-8%, 5 10% and 3-11% respectively (44, 73).

Therefore, despite the fact that masses of crops grow in the conditions of enhanced CO2 levels, the nutrient densities decrease. The availability of the soil nutrients is yet another source of complexity. In nutrient-rich alluvial soils, whose nitrogen levels do not change, rice covers become more enriched with nitrogen with higher CO2 levels, which counteracts the negative effect on the quality of grains (63). Conversely, on argillaceous soils, which have low nitrogen levels, especially in rain-fed rice monocultures in Africa, the carbon- nitrogen ratio widens significantly. In line with it, the levels of proteins in the grains decrease to the extent that endangers the necessary nutritional value to the populations that rely on them (17, 29). An examination of the worldwide literature indicates that there is a shortage of research that specifically highlights the deeper effect of the soil texture and fertility on the impacts of increased CO2 on rice nourishment

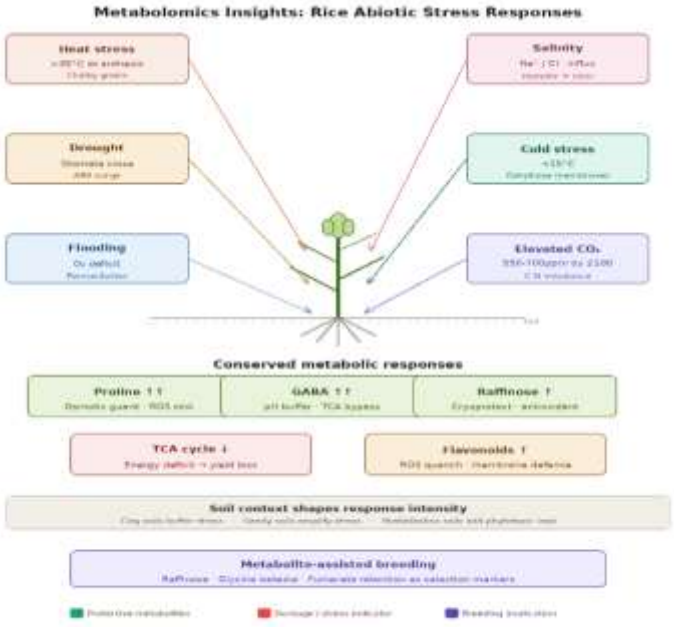

Figure 1. Overview of six major abiotic stresses affecting rice (Oryza sativa L.) and their predominant metabolic consequences. Arrows indicate stress-to-plant signaling; the lower panel summarizes metabolites consistently altered across multiple stress types

Table 1. Summary of Metabolic Responses to Each Abiotic Stress in Rice: Key Metabolites, Soil Context, Yield Effects, and Open-Access References

Stress	Key Metabolite Changes	Soil Modifying Factor	Yield / Quality Effect	Open-Access References
Heat	Proline↑, GABA↑, Raffinose↑, Flavonoids↑ Starch↓, Malate↓	N rich alluvial soils act as buffers to response; sandy soils increase the loss of TCA	A 20-30 % decrease in the weight of the grain is quite common and the grain becomes chalky with pollen sterile above 35 °C.	87, 24, 68, 6
Drought	Proline↑↑, Trehalose↑, Raffinose↑, BCAA↑ Glutamine ↓ Malate ↓	The process is quicker and steeper on a sandy or aerobic upland soil as compared to a clay Paddy soil	Losses of 20-40% have been observed in rain-fed systems; these are greatly due to a restriction in the ability to transport phloem assimilates, and resulting reduction in the number of grains	31, 8, 50, 75
Flooding	GABA↑↑, Ethanol↑, Alanine↑, lactate↑, Flavanoids ↑, Sucrose↓,	Clay soils extend anoxia; peat soils amplify secondary metabolites	When the adverse conditions for more than 10 days there could be a complete yield loss even when cultivars with the Sub1A gene suppress carbohydrate depletion	14, 4, 41, 79
Salinity	Proline↑, Glycine↑ betaine↑, Inositol↑, GABA↑, glutamine ↑	Clay CEC buffers Na^+; sandy soils accelerate ionic accumulation; alkaline soils add Fe stress	Pollen quality is lowered, 15-20% yield is affected and protein level in grains is increased while starch level is decreased	60, 47, 21, 92
Cold	Raffinose↑↑, Sucrose↑, Proline↑, PUFAs↑, Glutamate↓,	High-thermal-mass soils slow cold signal; thin/coarse soils amplify response	10-25% is reduced in seedling stage, 30-40% germination is failed below at 12 °C	89, 69, 91, 59
Elevated CO_2	Sucrose↑, Proline↑, starch ↑, Protein ↓ ,Zn, Fe ↓	N-rich paddy soils partially compensate C:N imbalance; N-poor soils amplify quality loss	10-20% yield is increased, 5-8% of protein is decreased , 3-11%, Fe 5-10%, Zn content in grain is reduced	44, 93, 1

3. Key Metabolic Pathways Reorganized Under Stress

Stress hits one cell part at a time, yet ripple effects travel across linked processes, sometimes magnifying or muting the first change. Among the six stress types examined, certain routes come up again and again - energy processing including the TCA cycle, building amino acids from nitrogen sources, the GABA detour in metabolism, along with making extras like those from the phenylpropanoid line. Connections between these paths - and their joint role during seed development - hold keys to reading chemical profiles in real crop results.

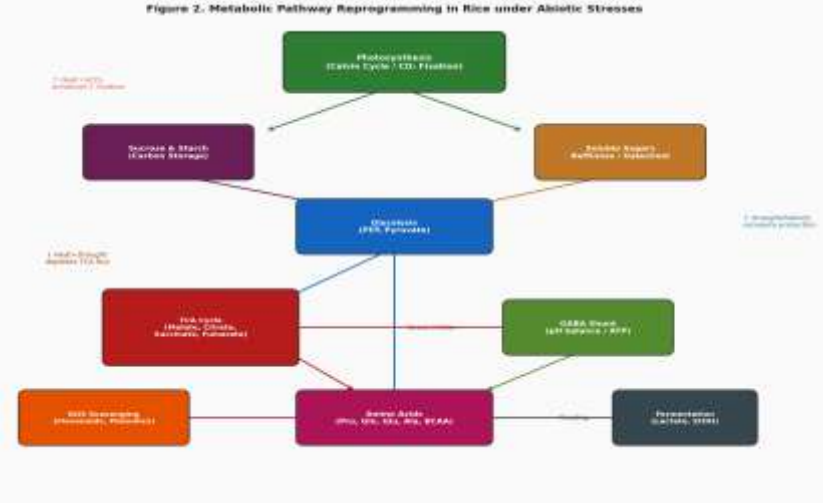

Figure 2. Metabolic pathway reprogramming in rice under abiotic stress. Green boxes indicate pathway upregulation; red boxes indicate suppression. GABA: γ-aminobutyric acid; TCA: tricarboxylic acid cycle; PEP: phosphoenolpyruvate; eCO_2: elevated CO_2.

3.1 TCA Cycle, Glycolysis, and Energy Supply

The main cause of the reduced performance of rice plants under abiotic stress is due to the loss of functions in tricarboxylic acid (TCA) cycle, which is a central energy generating pathway within the cell. Under high temperatures or limited water, intermediate metabolites malate, citrate, succinate, and fumarate decrease in concentrations to different degrees as either heat causes denaturation of enzymes directly or the lack of substrate due to drought. Therefore at a time when the plant needs its capacity to produce adenosine triphosphate (ATP)-dependent processes such as phloem loading of sugars, extrusion of toxic sodium ions, and protein repair by reactive oxygen species are most needed are impaired. Thus, the deficiency is not the deficiency of raw carbon constituents but the deficiency of the energy fuel needed to mobilize and distribute the carbon constituents.

During flood conditions, cellular glycolysis continues but instead of converting pyruvate to acetyl -coA and enters the TCA cycle, it diverts to fermentative pathways producing ethanol and alanine (4, 14). The pentose phosphate pathway gets more pronounced under saline or drought stress and offers reducing power through NADPH and ribose-5-phosphate to disrupt antioxidant regeneration through glutathione and activation of stress-responsive genes (16). These alternative pathways, which are adaptive in times of acute stress, redirect carbon flow into the standard TCA pathway leading to a reduced ATP production, further limiting energy availability requirements during grain development.

3.2 Nitrogen Metabolism: GS/GOGAT Cycle and Amino Acid Dynamics

In rice absorption of inorganic nitrogen occurs only via the glutamine synthetase-glutamate synthase (GS/GOGAT) pathway, where ammonia is incorporated into glutamine through glutamine synthetase and the amide nitrogen base to glutamine through glutamate synthase (31, 82). Water scarcity inhibits this pathway by reducing the uptake of nitrogen by roots, salinity interferes with this pathway through competitive inhibition of ammonium transporters by sodium ion, and increased atmospheric CO2 concentrations disrupts the anabolic balance by saturating the system with surplus carbon that is not balanced by nitrogen concentrations. The net effect is that it reduces glutamine levels leading to down-regulation of amino acid synthesis downstream. The inability of glutamine results in a metabolic diversion in the cell. Branched-chain and aromatic amino acids are released through proteolytic degradation, their carbon skeletons are used in the TCA cycle in place of glutamine. At the same time, there is proline accumulation as glutamate is redirected to produce this multifunctional amino acid, which helps in osmotic homeostasis, stabilization of proteins, and reactive oxygen species scavenging (28, 72). The result is a reproducible signature, that is, an increase in branched-chain amino acids and a decrease in glutamine and asparagine, a spike in proline and a corresponding decrease in TCA intermediates. Such changes are adjusted by the kind and intensity of stress but otherwise do not change throughout the range of abiotic stresses facing rice

3.3 GABA Shunt and Secondary Metabolite Defence

When oxygen is depleted and flooding occurs, the γ -aminobutyric acid (GABA) shunt is used to bypass a very oxygen-sensitive portion of the tricarboxylic acid (TCA) cycle. Fortunately, instead of concluding at the 2 -oxoglutarate dehydrogenase complex, this other pathway follows the conversion of glutamate to succinic semialdehyde through GABA, thus continuing to support a carbon flux that is independent of an intensive oxidative phosphorylation (43, 77). Flooded cells therefore can proceed to feed the TCA cycle despite the blockage of respiratory routes. There is extra stress on the buffer of intracellular pH due to higher temperatures, which also help in buffering intracellular pH as the GABA shunt takes protons during the process of catabolism of glutamates. Activation of this metabolic pathway is repeatedly evoked in rice in a variety of stress situations such as salinity, heat, waterlogging, and a combination of them, and hints at the strongest involvement in the situation when mitochondrial homeostasis is destabilized. A second major branch of chemical defence made by phenyl propanoid derivatives of metabolites, especially flavonoids and hydroxylcinnamic acid derivatives, is invariably induced during a variety of stress conditions in rice. Their transcriptional up-regulation is

causally connected to the production of reactive oxygen species (ROS): exposure of electron-transport chains in chloroplasts or mitochondria to overloading under heat, flooding or combined stresses leads to an oxidative burst which in turn activates the expression of phenylalanine ammonia-lyase (PAL) and the downstream synthetic enzymes.
These flavonoids and phenolic acids generated have both anti-singlet oxygen quenching/anti-hydroxyl radical quenching capabilities, excess photosynthetically active radiation takeovers, and reinforcement of cell-wall polymers to restrict the harmful effects of membrane damage. The redirection of phenylalanine and carbon skeletons by the production of phenylpropanoids, although indirectly, at the expense of primary metabolism is compensated by its constant and reliable induction in the face of various stressors in rice tissues, thus highlighting the necessity of its essential importance to primary metabolism. It is noteworthy in particular that a sharp increase of flavonoid concentration takes place at the post-submergence phase of re-aeration, at the very time when the reestablishment of aerobic respiration leads to an ROS burst which, unless counter measured, would otherwise lead to massive membrane lipid peroxidation (46, 85).

Figure 3. Heatmap of metabolite response profiles across six abiotic stresses in rice, compiled from GC-MS and LC-MS studies. ↑↑ strong increase; ↑ moderate increase; — no change; ↓ moderate decrease; ↓↓ strong decrease.

Table 2. Major Metabolic Pathways Altered by Abiotic Stress in Rice: Enzymes, Directional Changes, and Functional Consequences

+Pathway	Key Enzymes	Stress-Induced Direction	Metabolic Consequence and References
TCA Cycle	Aconitase, MDH, SDH, ICDH	↓ under drought , heat , salinity	Reduced ATP synthesis; faulty phloem loading; and a sharp drop in weight of grain (49, 87).
GABA shunt	SSADH, GAD, GABA-T	↑↑ under flooding and in combined stresses	Cytosolic pH buffers ; TCA acts as a bypass in hypoxia condition and maintaining carbon flux (57)
GS/GOGAT cycle	Fd-GOGAT, GS1, GS2	↓ under salinity , drought ,eCO2	Causing C:N imbalance; lower grain protein; proline accumulates as N overflow (31, 82)
Proline biosynthesis	ProDH, P5CS, P5CR	↑↑ under all major stresses	Helps in osmotic adjustment and removal of ROS and a redox sink (28, 72).
Raffinose pathway	Rafs and GoIs	↑ under heat, cold, drought	Which offer membrane cryoprotection, antioxidant activity enhancement, and carbon redistribution (20, 61).
Starch biosynthesis	GBSS, SSI, SSIIa	↓↓ under heat and flooding	leads to chalky grains development , reduced endosperm starch, and direct yield loss (68, 87)

Phenylpropanoid /Flavonoid	CHS, F3H, PAL and DFR	↑ under all stresses (ROS-driven)	It provides membrane and antioxidant defence, post flood re-aeration quenching ROS (46, 85)
Fermentative pathways	ADH, LDH, PDC	↑↑ under flooding/ anoxia	Production of alanine and ethanol; depletion of carbohydrate pool (4, 41).

4. Metabolic Responses to Combined Abiotic Stresses

One of the most important lessons coming out of recent multi-stress metabolomics work in rice is that stress combinations produce responses that cannot be predicted by simply summing up the effects of individual stresses. The technical term is non-additive metabolic interaction, and it matters enormously for connecting laboratory data to field performance because in farmers' fields, stresses combine constantly (42, 71). A heat wave during the dry season creates drought simultaneously. Coastal flooding events carry salinity. Rising CO_2 is always present as a background condition modifying how plants respond to heat or drought. Understanding these interactions is not an academic refinement; it is a prerequisite for accurate prediction of how rice metabolizes under real agricultural conditions.

4.1 Heat Combined with Drought

Heat and drought occur concurrently, which means it has the most metabolically hard condition ever documented in rice. Under this dual strain, routinely, proline accumulation is observed to reach 15-25 fold increases over baseline, which is significantly greater than increases of 3-8 fold in either of the two singular conditions, indicating the combined osmotic adjustment and thermal denaturation protection of proteins in the plant (33). Glutamic acid- γ- aminobutyric acid (GABA) also increases 4 -6 fold, which is a sign of exaggerated tricarboxylic-acid-cycle disruption and cytosolic pH-distortion beyond the effect that either stress alone produces. Raffinose and galactinol coexist synergistically in the reproductive organs with a resultant effect of stabilizing the membranes during the accumulated attack on the pollen and the endosperm development.

The tricarboxylic -acid cycle almost fails during the combined heat-drought condition that is even more severe than their respective stressors, thus creating an ATP deficit in spikelets struggling to provide energy to biosynthesize osmolytes, restore membranes, and mature reproductively (5, 34). Therefore, under this combined pressure, the metabolic trade-off between protection compound biosynthesis and the effective allocation of carbon to grain filling achieves its most deleterious extreme, explaining why yield losses due to heat-drought at anthesis regularly exceed 50% and significantly the losses predicted by either of the two stresses alone. This finding highlights the inefficiency of individual stress paradigms in the comprehension of the comprehensive level of physiological imbalance that combined heat and drought cause.

4.2 Drought with Elevated CO_2

The interaction of drought stress and high CO2 levels is a form of partial metabolic antagonism. High CO2 causes a higher efficiency of using water and maintains photosynthetic carbon supply during mild drought and partially represses the abscisic acid-regulated metabolic programme of drought stress; the accumulation of branched-chain amino acids and the formation of antioxidant substances are decreased compared to standard drought (35, 39). Even though this suppression seems to be advantageous, it is not accompanied by an equal increase in stress protection, which suggests that the plant is less metabolically adapted to the drought it goes through.

The carbon-to-nitrogen ratio triggered by high CO2 is further exaggerated in the state of drought, with the decrease in the mass of roots reducing nitrogen uptake, but carbon fixation is preserved by high CO2 (44, 82). Combined drought and increased CO2 cause rice cultivars to have insufficient protective amino-acid reserves, and inadequate nitrogen (grain) content in the grains, a two-way nutritional trade off. This combination is expected to be more widespread in the rain-fed tropical rice systems in the late-21 st century climate conditions.

4.3 Flooding Combined with Salinity

Onshore rice zones affected by tidal intrusion or seawater flooding caused by cyclone are affected by hypoxic and ionic stress operating simultaneously and the metabolic interaction is synergistic and not additive (54). The concentrations of fermentation metabolites are two or three times higher than those observed when only flooding is present, due to the energy expense of Na^+ exclusion systems reducing carbohydrate reserves, which would otherwise support less vigorous fermentative activity (15, 60). This combined stress reaches its highest known levels of GABA, which acts as both an anaerobic carbon-flux pathway and a cytosolic pH buffer to fermentation acidosis and Na^+ influx acidification.

Saline post-flooding recovery is significantly slower and more metabolically active when compared to the recovery of flooding alone (4, 79). Re-aeration reactive oxygen species burst is more pronounced, accumulation of flavonoid and phenolics in recovery is enhanced and reserves carbohydrates needed to resume aerobic metabolism and active Na^+ exclusion are more depleted. This is the reason why field recovery of rice after saline flooding is much worse than that of freshwater flooding despite the fact that inundation period is kept constant.

Table 3. Non-Additive Metabolic Responses to Combined Abiotic Stresses in Rice

Stress Combination	Interaction Type	Distinctive Metabolic Signature	Yield Outcome and References

Heat + Drought	Synergistic	Proline ↑↑↑ (up to 25×), GABA ↑↑, TCA collapse, Raffinose ↑↑, ATP deficit	Up to 50% yield loss at anthesis; exceeds sum of individual effects (33)
Drought + eCO2	Partially antagonistic	BCAA↓ compared to those in drought ; C:N is increased, drought induced signalling is partially suppressed	Presence of eCO2 seems to suppress the readiness of plant to drought . As a result the quality of nutrition is decreased (35
Flooding + salinity	Synergistic	Fermentation metabolites ↑↑↑, GABA ↑↑↑, depletion of sucrose is increased faster , Concentration of ROS is increased ↑↑ at recovery	The combined stress leads to slower, incompletely resolved recovery compared with flooding alone, accompanied by profound carbohydrate depletion (4).
Heat + eCO_2	CO_2 benefit eliminated by heat	Sucrose ↑ initially then feedback-suppressed; starch biosynthesis still collapses; Proline ↑	Net yield effect neutral to negative; eCO_2 fertilization advantage cancelled (1)
Salinity + Drought	Additive–Synergistic	Proline ↑↑, glycine betaine ↑, GABA ↑, glutamine ↓↓	Severe metabolic burden on roots; 30–50% yield penalty in susceptible lines (21, 47)

5. Evidence from Single-Stress Metabolomics Studies

The metabolic fingerprints described in Sections 2 through 4 were built almost entirely from single-stress experiments: growth chamber studies where temperature, water, salt concentration, or CO_2 level was altered in isolation while everything else was held constant. These studies are genuinely foundational. They assessed which metabolites reacts to which stresses, determined the paths that are involved illustrated the differences between the genotypes that are susceptible and tolerant, and created the potential biomarkers that are currently being tested in breeding programs.

Several representative metabolomics studies have provided experimental evidence supporting these stress-specific metabolic responses. Among them, (87) made a ground breaking study of the metabolomics changes in heat stress at the grain level which not only gave unparalleled resolution but also shown a cause and effect relationship between starch enzymatic dysfunction and the occurrence of the chalky grain phenotype. In a thorough synthesis of GC -MS based literature related to rice responses to abiotic stresses such as heat, drought and salinity, (49) substantiate the universal stress biomarkers proline and γ -aminobutyric acid (GABA) with consistent logical rationale of coherent metabolic pathways. (8) described the genotypic differences in drought responsive metabolite profiles of a genetically diverse rice panel, which uncovers that changes in proline, trehalose and raffinose concentrations offer better predictive measures of drought tolerance as compared to the traditional physiological measures. These foundational studies are what gave metabolomics its credibility as a rice stress biology tool.

The metabolic basis of Sub1A-conferred submergence tolerance was determined in (14, 23), which revealed that the metabolic method of conserving the use of a simple carbohydrate, fermentation inhibition, is more effective than the carbohydrate depletion that occurs during submergence.(60) presented the most conclusive metabolomic separation between tolerant and susceptible rice hybrid under salinity stress, and identified glycine betaine and inositol as the most discriminative values of the tolerant phenotype. (89) and (20) also recorded the response of the raffinose family oligosaccharide under a range of cold-tolerant genotypes in the context of cold stress, whereas (69) clarified the evolution of the cold metabolome throughout developmental stages in a way that was relevant to the breeding selection timing.

The metabolic territory explored in section 4 – the synergistic proline surges, TCA collapses and antioxidant responses which arise from stress combinations is what single stress studies have failed to capture . The TCA breakdown under combined heat-drought is not predictable from the individual stress profiles (33). The suppression of drought metabolites by concurrent eCO_2 is entirely invisible in single-stress drought studies.. Single-stress data remains essential context, but its limitations as a guide to field metabolic behaviour are now well enough established to be treated as a core design consideration for future research.

6. Knowledge Gaps, Future Directions, and Breeding Implications

6.1 Field Metabolomics and Temporal Resolution

The most visibly adverse constraint of the existing rice metabolomics literature, perhaps, is its limited extent of being carried out in real settings in the real stress situations. The logistical impediments exist in reality - anther and young grain tissues exhibit that turnover of key metabolites in a few hours of stress onset, and harvesting them out of a flooded paddy or a heat-impacted field without frozen in liquid nitrogen results in the degradation of metabolites before it can be analysed (33, 49). This means that the bulk of the understanding researchers have of stress metabolomics in rice is based on systems where stress was induced in controlled conditions on plants planted in pots or hydroponic culture - the situations that

eliminate the soil heterogeneity, microbes-host interactions, and changing stress levels that typify real agricultural conditions.

The development of portable LC-MS systems and enhanced field-deployable cryo-sampling criteria is starting to render real-time metabolite profiling in the field of use manageable (33). Such tools, used in a systematic way, in other words, at key reproductive stages during natural stress events in a variety of soil types and seasons, will probably uncover metabolic processes that have never been revealed in the controlled experiments. There is also a requirement of the time-course studies that monitor the changes in the metabolites over the stress onset, peak, and recovery, instead of the end-point level, to determine the metabolic momentum leading to tolerance or susceptibility.

6.2 Multi-Stress Designs and Systems-Level Analysis

Factorial multi-stress experiments that compare pairwise and three-way stress combinations at essential developmental points are the single highest priority gap in the approach of rice stress metabolomics methodology. What the few multi-stress studies available to us have learned, especially the GABA shunt activation when it is subjected to combined heat-drought (33) and the C:N imbalance amplification when it is subjected to drought-plus eCO2 are a very powerful indication that we are operating in an imperfectly descriptive picture of the reality of how rice actually metabolizes in a realistic climate situation. These pictures would be supplemented with quantitative precision by metabolic flux analysis with stable isotope labelling and constraint-based network modelling to understand the movement of carbon through pathway networks in the face of combined stresses instead of the changes in pool sizes

6.3 Metabolite Biomarkers for Breeding

A number of metabolites now have sufficient supporting data using independent studies and varied genetic backgrounds to be suggested as believable biomarker outcomes in stress tolerance selection in rice breeding programmes. The oligosaccharides of the raffinose family, especially raffinose and galactinol are also found to be uniformly associated with heat, cold, and drought-tolerance across the various genotype panels (61). Heat-drought fumarate retention has become a predictor of functional maintenance of TCA cycle and linked with the enhanced stability of yield in multi-stress studies. A demonstrable salinity tolerance marker that is used in glycine betaine accumulating varieties is glycine betaine (21, 60). Proline is almost universal but predictive power in regard to tolerance vs susceptibility is dependent on the genotype situation and also the rate oaccumulation and not the absolute level.

Genetic architecture of these metabolite characteristics is now made available in metabolome-wide association studies (mGWAS) and metabolite QTL (mQTL) mapping. The association between the drought proline accumulation and the locus of the DREB-family transcription factors have been validated in thepopulations of rice (70). The use of metabolite-based selection criteria as well as phenotypic traits in multi-environment breeding trials is a rational step that has not yet been carried out by most groups of metabolomics research. The metabolomics laboratories will need to collaborate with plant breeding programmes, at least those with FACE facilities or with multi-stress field nurseries, to test biomarkers at the scale they make a significant contribution to the decision to select varieties (30, 51).

Table 4. Knowledge Gaps in Rice Stress Metabolomics and Proposed Research Approaches

Knowledge gap	Current limitation	Proposed approach and references
Field based metabolomics	Most of the research data comes from controlled environments ; Transient dynamics and soil variability data is missing	Portable LC-MS instruments in the field followed by cryo field sampling methods and conduct multi season studies to capture real time environmental variations (33)
Multi stress experimental design	Many studies focus on single stress however in real field conditions plants face multiple stresses at the same time so this combined effect of stresses different from single stresses	Factorial 2×2 and 3-way to study stress experiments at stages of anthesis and grain filling; methods like stable isotope flux can help understand plant responses under combined stress condition (42)
mQTL / mGWAS for tolerance	Current metabolomics studies limited germplasm diversity in rice populations because of this many genes related to stress tolerance is not identified	Future research should focus on including diverse rice varieties and wild oryza species and perform multi stress metabolite trait mapping to identify the new tolerance genes (70)
Soil × metabolome interaction	Many studies do not properly report the soil type even the soil conditions affect the plant metabolism also the rhizosphere metabolomics is rarely studied	Future research should focus on how to analyse different plant metabolites in different a soil types , study root exudates and combine metabolomics with soil microbiome data to better understand of plant-soil interaction (10, 27)
Biomarker validation in breeding	Some studies have identified metabolite biomarkers for stress	To make these markers useful for crop improvement they should do multi location

	tolerance but they rarely conducted in large field trails	field experiments and included in metabolomics assisted breeding selection methods (60, 61)

7. CONCLUSIONS

The rice stress metabolomics in totality is a more complex story than the number on the headline of yield loss due to heat or drought might suggest. Stress does not merely kill plants, but restructures them, occasionally to the benefit of the most vital functions whilst also reducing the total growth. It is important to understand that what metabolomics can provide is reorganization in metabolic terms and the image that has been created with the help of the reviewed literature is detailed enough to make some quite confident conclusions as well as some candid admissions of what is yet to be known.

A number of metabolic themes have now been developed to a degree of being considered as core knowledge. The oligosaccharides of proline, GABA and raffinose-family accumulate under all major abiotic stress in rice, albeit by slightly different causes, through various triggers based on the nature of the stress. TCA cycle inhibition is a valid indicator of metabolic seriousness and a direct reason of yield loss by energy restriction. Hyper extensive thermos sensitivity of enzymes in the synthesis of starch causes starch biosynthesis to fail at room temperature in a manner that cannot yet be counteracted by any known metabolic supplement. High CO2 produces C:N imbalance decreasing the nutritional value of grains without always causing the protective metabolic action of the classical abiotic stresses. And interactions between stresses have non-additive effects on metabolism which are not predictable by single-stress experiments.

The practical implication of all this on the rice improvement is that metabolomics is no longer a descriptive tool. Metabolite characteristics, raffinose accumulation, fumarate retention under combined stress, glycine betaine under salinity, and others, are satisfying the criteria of metabolite-assisted selection: they are measurable, they have a heritable component, and they are mechanistically relevant, and in all genotypes they are concurrently associated with stress tolerance. The discrepancies between these data and their everyday applications to breeding pipelines are still vast, although it is becoming smaller as sources of mQTLs continue to grow and as laboratory capability is matched by infrastructure in the field. Sealing that innermost gap, as well as developing multi-stress and field-based metabolomics, is the most obvious way out of the biochemical knowledge examined in this paper to rice varieties that can actually supply the food under the climate conditions that are already coming.

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