Metabolomic Insights Into Maize Salt Stress Response and Tolerant Genotypes

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Abstract Soil salinity operates as a worldwide abiotic stress which restricts maize (*Zea mays* L.) cultivation and threatens worldwide food security. Recent studies in metabolomics have enabled scientists to identify the biochemical and molecular pathways which control salt tolerance in maize plants. The research shows that tolerant genotypes produce more osmoprotectants including proline and raffinose and soluble sugars and secondary metabolites like flavonoids and phenolic acids which work together to protect cells through osmotic adjustment and ROS detoxification and membrane stability. Studies of networks demonstrate that tolerant genotypes form modular structures with redundant components which enhances their resistance to stress. The combination of metabolomics with quantitative genetics through mQTL and mGWAS methods has discovered specific biomarkers and causal genes which include proline and raffinose and lipid metabolism genes that can be used directly for breeding purposes. Functional validation using transgenic and genome editing technologies confirms causal links between metabolites and salt resilience. Metabolite markers show their translational value for germplasm screening and breeding pipelines through particular examples. The future of metabolomics will experience a transformation through spatiotemporal metabolomics advancements and multi-omics integration and computational modeling which will shift the field from descriptive observation to predictive and mechanistic biology. Scientists use Metabolomics as a strategic platform to study salt tolerance mechanisms while creating salt-resistant maize varieties for sustainable salt-affected area agriculture.

Keywords Maize (*Zea mays* L.); Salt stress; Metabolomics; Osmoprotectants and secondary metabolites; Molecular breeding

1 Introduction

Soil salinity is a major abiotic stress threatening global agriculture, with nearly 20% of irrigated land worldwide already affected and the extent projected to expand due to climate change and intensive irrigation. Maize (*Zea mays* L.) ranks as the world's third most important cereal crop yet it remains highly sensitive to salt stress which causes growth reduction and decreased grain yield and quality during its early developmental phases. This makes salt tolerance improvement an urgent priority for global food security and sustainable agriculture.

Traditional physiological research into maize salt tolerance has developed into modern scientific studies which use molecular and omics-based approaches. The first studies investigated how sodium and chloride accumulation results in cell damage through osmotic disruptions and toxic ion effects but contemporary molecular studies have discovered stress response pathways which involve ion transporters and osmoprotectant synthesis and transcriptional regulation. High-throughput omics technology has made metabolomics a strong analytical method to study salt tolerance mechanisms through its ability to show how genomic and transcriptomic and proteomic regulations combine (Brar et al., 2025; Ren et al., 2025).

The tolerant maize genotypes produce elevated amounts of protective metabolites which include proline and flavonoids and fatty acids that help with osmotic adjustment and antioxidative defense and membrane stability under salinity conditions (Yue et al., 2020; Khan et al., 2024). The metabolic pathways that undergo reprogramming include starch and sucrose metabolism and amino acid biosynthesis and phenylpropanoid metabolism which help plants adapt to stress conditions (Ren et al., 2025). The research investigation identified three genes which show promise for molecular breeding because they connect to plant salt tolerance mechanisms through citrate synthase and glucosyltransferases and cytochrome P450s (Liang et al., 2021; Brar et al., 2025).

The research findings expand our understanding of maize stress biology while delivering useful biomarkers which can be used for marker-assisted selection and genomic prediction. The main goals of this review consist of three parts which include (i) explaining maize responses to salt stress at both physiological and molecular levels (ii) combining metabolomics data to understand salt-sensitive pathways and genetic variations between different maize genotypes (iii) and applying metabolomic results to develop breeding approaches for salt-resistant maize varieties.

2 Physiological and Molecular Basis of Salt Stress in Maize

2.1 Salt stress simultaneously induces ion toxicity and osmotic imbalance

The main effects of salt stress on maize plants occur through two mechanisms which combine osmotic stress with ion toxicity. The excessive sodium ions (Na^+) in rhizosphere space compete with potassium (K^+) to enter plant cells which causes problems with K^+-dependent processes that include enzyme activation and protein synthesis and cell turgor pressure regulation (Zhang et al., 2018; 2019; Cao et al., 2023; He et al., 2025). High blood Na^+ concentrations lead to membrane instability because the ions force Ca^{2+} out of membrane surfaces which results in damage to both membrane structure and signal pathways (Zhang et al., 2019; 2025).

The combination of ionic disturbances with decreased soil water potential under saline conditions leads to drought-like stress which prevents root water absorption while causing stomatal closure and decreasing transpiration and photosynthesis (Ji et al., 2025). The leaf wilting and chlorosis and growth reduction of sensitive maize genotypes differ from tolerant genotypes which use Na^+ exclusion from shoots and vacuolar compartmentalization and osmotic adjustment to maintain cellular homeostasis (Zhang et al., 2018; Wang et al., 2022).

2.2 Excessive accumulation of reactive oxygen species necessitates antioxidant defense and signaling

Salt stress causes plants to produce excessive reactive oxygen species (ROS) including superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\cdot OH$) in addition to ionic and osmotic stress. The ROS production happens in different organelles such as chloroplasts when electron transport is disrupted and in mitochondria through respiration changes and peroxisomes during photorespiration (He et al., 2023; Yan et al., 2025). ROS function as signaling molecules at moderate concentrations to trigger stress-response pathways but their excessive accumulation beyond antioxidant defenses results in oxidative damage which produces lipid peroxidation and protein carbonylation and DNA fragmentation (Ji et al., 2025). The enzymatic antioxidants superoxide dismutase (SOD) and catalase (CAT) and ascorbate peroxidase (APX) along with non-enzymatic antioxidants ascorbate and glutathione and flavonoids help maize plants fight oxidative stress. The tolerant genotypes show higher basal antioxidant levels and fast antioxidant defense activation which leads to superior protection against oxidative damage (He et al., 2023).

2.3 Phenotypic divergence between tolerant and sensitive maize genotypes reflects underlying molecular regulation

Salt tolerance in maize produces multiple visible traits which result from intricate genetic control systems. The tolerant genotypes outperform sensitive genotypes in water content preservation and photosynthesis and membrane protection according to Ji et al. (2025). Root system architecture functions as a key factor because plants with deeper or more branched root systems can better absorb water under osmotic stress conditions (Cao et al., 2023).

The molecular basis of salt tolerance in tolerant lines involves changes in the expression of Na^+ transporter genes HKT and NHX and SOS families as well as osmoprotectant biosynthesis genes proline synthase and transcription factors AP2/ERF DREB NAC bZIP WRKY and MYB (Zhang et al., 2019; Zhou et al., 2022; Yan et al., 2023; Zhang et al., 2025). The process of salt tolerance development in maize involves epigenetic modifications including DNA methylation and histone acetylation which regulate gene expression and the newly discovered genes ZmHKT1, ZmHAK4, ZmSOS1, Zm4CL8 and ZmESBL show potential for salt tolerance breeding according to Wang et al. (2022), Zhang et al. (2018; 2025).

3 Metabolomics Approaches in Salt Stress Research

3.1 Advanced analytical platforms enable comprehensive profiling of salt-responsive metabolites

High-resolution and high-throughput analytical platforms function as essential equipment for scientists to study all biochemical reactions that occur in maize plants when they experience salt stress. The three methods for metabolite analysis consist of GC-MS and LC-MS and UPLC-QTOF-MS. The LC-MS method stands as the primary choice because it offers superior sensitivity and identifies numerous metabolites. The UPLC-QTOF-MS system delivers the highest resolution and precise mass detection capabilities for identifying both primary and secondary metabolites. GC-MS maintains its position as the top method for detecting volatile compounds and derivatized polar metabolites including organic acids and amino acids and sugars. The low sensitivity of NMR spectroscopy does not reduce its value because it provides absolute metabolite quantification with high reproducibility and quantitative accuracy.

The field of metabolomics has achieved better resolution through three new methods: direct-infusion MS (DIMS) performs rapid metabolite detection and ion mobility spectrometry–MS (IMS-MS) separates identical metabolites and imaging MS produces tissue maps that help researchers study salt stress effects on root and leaf and reproductive organ heterogeneity. The platforms function as a unified system which provides superior metabolome analysis through broader coverage and improved analytical accuracy (Xia and Wishart, 2016; Pang et al., 2024).

3.2 Multivariate statistics and pathway enrichment reveal stress-responsive patterns

The analysis of raw metabolomic data requires powerful statistical and bioinformatics methods because these datasets contain numerous thousands of features that change between different genotypes and treatment conditions and developmental phases. The unsupervised analysis of global metabolic changes becomes possible through principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) allows supervised discrimination between tolerant and sensitive genotypes based on metabolic signatures. The most affected metabolites become evident using univariate methods in combination with volcano plots.

The KEGG and Plant Metabolic Network (PMN) pathway enrichment analysis shows that amino acid metabolism and glycolysis and flavonoid biosynthesis pathways receive the most substantial changes from these modifications. Network-based approaches such as weighted correlation network analysis (WGCNA) further reveal co-regulated metabolite clusters tightly associated with salt tolerance traits (Chong et al., 2018; Pang et al., 2024). The end-to-end workflow of MetaboAnalyst and metaX software platforms includes preprocessing and normalization and multivariate statistics and pathway analysis functions while PhenoMeNal operates as a cloud-based system for large-scale metabolomics studies (Wen et al., 2017; Sun and Xia, 2023).

3.3 Multi-omics integration enhances mechanistic understanding

The interpretation of biochemical end-products from cellular regulation becomes more straightforward when metabolomics data is integrated with information from other omics layers. Scientists use metabolite and transcript relationship analysis to understand transcriptional control of metabolic pathways by studying cases such as salt-stressed tolerant maize which produces more proline through elevated P5CS (Δ^1-pyrroline-5-carboxylate synthase) gene expression. The combination of proteomics with enzyme abundance analysis allows scientists to investigate metabolic flux and ionomics delivers extra data about ionic equilibrium (Chong et al., 2018).

The research on maize salt stress now uses two systems biology frameworks which include genome-scale metabolic models (GEMs) and metabolic flux analysis (MFA). The models use multi-omics data integration to forecast how metabolic fluxes change under stress conditions which enables researchers to connect genetic information with metabolic responses and adaptive results. The integration of multiple omics approaches enables researchers to move beyond descriptive biomarker analysis of metabolomics data which results in mechanistic knowledge about salt tolerance in maize.

4 Key Metabolic Pathways in Maize Salt Stress Response

4.1 Osmoprotectants and compatible solutes mediate osmotic adjustment

The research on maize under salt stress conditions shows that osmoprotectants known as compatible solutes accumulate as a primary response. Under salinity conditions proline functions as an osmotic buffer and membrane stabilizer and ROS scavenger and protein stabilizer which leads to its strong induction. The proline content of tolerant maize genotypes shows higher levels at both resting state and under stress conditions than sensitive genotypes (Li et al., 2021; Shoukat et al., 2024). The osmotic regulator glycine betaine exists at low concentrations in maize plants yet protects photosystem II and functions as an osmotic regulator while tolerant or transgenic lines with elevated synthesis levels show enhanced salt tolerance (Liang et al., 2021; Hou et al., 2024).

The osmotic adjustment process depends on soluble sugars including glucose, fructose, sucrose, raffinose and trehalose which serve to protect against oxidative stress and act as stress signaling molecules (Luo et al., 2018; Hou et al., 2024; Wu et al., 2024). RFOs serve as osmolytes and antioxidants which protect cells from osmotic and oxidative stress through their dual function. The application of nano-fertilizers results in enhanced sugar metabolism and better ionic equilibrium in maize plants that experience salt stress according to Shoukat et al. (2024).

4.2 Secondary metabolites contribute to ROS scavenging and stress signaling

The salinity stress causes significant changes in secondary metabolism which leads to increased production of flavonoids and phenolic acids and terpenoids. Flavonoids contain antioxidant properties which defend chloroplasts and membrane structures from ROS-induced damage. The tolerant genotypes build up Quercetin and kaempferol and luteolin and their derivatives which leads to better resistance against oxidative stress (Ullah et al., 2025). Plants obtain protection from oxidative stress through phenolic acids such as ferulic acid and caffeic acid which also help build lignin for stronger cell walls during stressful periods.

Terpenoids which include diterpenes and sesquiterpenes function as signaling compounds to link stress responses with hormonal pathways. Plants generate terpenoids during salt stress to regulate the ABA-JA and SA signaling pathways which link metabolic defense mechanisms to signaling pathways (Ullah et al., 2025). The antioxidant defense system of secondary metabolites in maize operates through signaling pathways to generate a single response against salinity stress.

4.3 Remodeling of energy and lipid metabolism sustains cellular homeostasis

Salt stress causes major changes to both central carbon metabolic pathways and lipid structural composition. The cellular processes of glycolysis and tricarboxylic acid (TCA) cycle function as stress adaptation mechanisms which maintain energy supply stability. The cell generates ATP and reducing power through enhanced glycolysis which allows it to produce osmoprotectants and transfer nitrogen through pyruvate and malate intermediates (Li et al., 2021; Wu et al., 2024).

The body depends on lipid metabolism as a vital process to preserve membrane stability when cells experience saline conditions. Salt stress activates fatty acid desaturation which results in better membrane fluidity and stability. The process of phospholipid remodeling results in phosphatidic acid and inositol phosphates formation which serve as secondary signaling molecules during stress responses. Polyunsaturated fatty acid-derived oxylipins including jasmonates activate defense mechanisms through their role as signaling molecules (Luo et al., 2018). The tolerant genotypes maintain superior lipidomic flexibility for membrane stability and stress signaling when exposed to salinity conditions (Wu et al., 2024; Ullah et al., 2025). Based on combined metabolomics and transcriptomics analysis, Liang et al. (2021) constructed an integrated model of maize salt stress response, revealing the coordinated regulatory relationships between metabolic pathways (Figure 1). This model is of great value in understanding the mechanisms of maize salt tolerance.
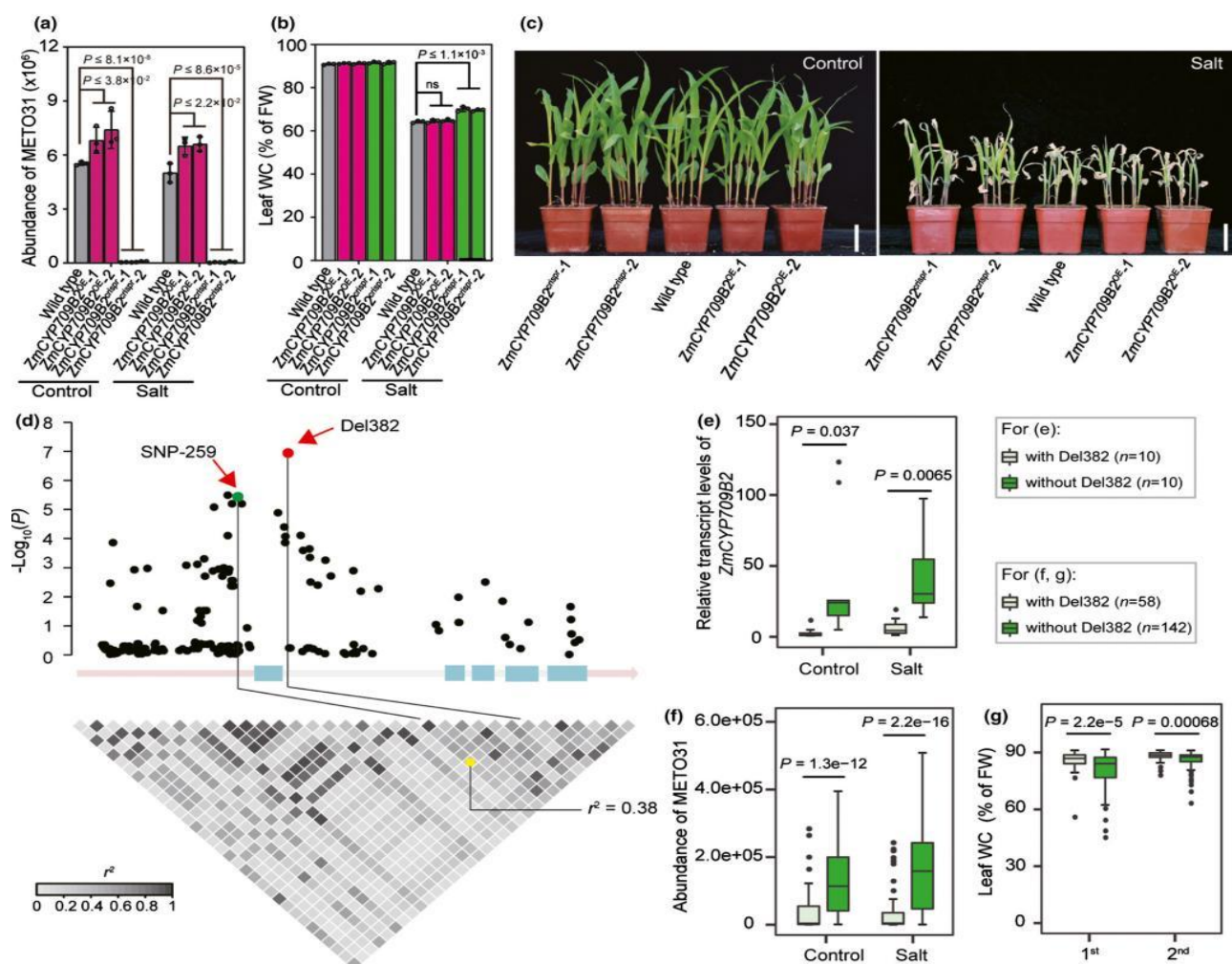
Figure 1 Integrated model of salt stress response in maize revealed by metabolomic and transcriptomic analysis (Adapted from Liang et al., 2021). The schematic summarizes osmoprotectant accumulation, ion transport, and signal transduction pathways that coordinate salt tolerance in maize

5 Comparative Metabolomics of Tolerant vs. Sensitive Genotypes

5.1 Differential metabolite accumulation patterns highlight adaptive strategies

The research studies using comparative metabolomics show that salt-resistant maize varieties produce more osmoprotectants including proline and sucrose and raffinose and antioxidant compounds like flavonoids and phenolic acids than salt-sensitive varieties which do not develop protective metabolic responses for osmotic balance and ROS detoxification (Liang et al., 2021; Zhao et al., 2023; Brar et al., 2025). The tolerant lines show elevated proline content at both rest and stress conditions and RFOs function as protective agents through their roles as osmolytes and antioxidants. Research studies have found three metabolites that show differences between tolerant and sensitive lines because of their distinct resource allocation patterns (Liang et al., 2021; Zhao et al., 2023). The different accumulation patterns demonstrate that tolerant maize plants focus on maintaining osmotic balance and ROS detoxification and metabolic resilience during salinity stress. The metabolomics analysis by Brar et al. (2025) showed that there were significant differences in the accumulation of proline, raffinose and flavonoid metabolites between salt-tolerant and sensitive maize genotypes (Figure 2), which constituted the metabolic basis for their adaptation to salt stress.

5.2 Network robustness underpins resilience in tolerant genotypes

The distinction between tolerant and sensitive maize genotypes depends on both individual metabolite variations and network-level differences. The tolerant lines show more modular interconnected and redundant metabolic networks which have multiple pathways including proline GABA and polyamines that work in parallel to reduce osmotic and oxidative stress and provide robustness through redundancy (Liang et al., 2021; Brar et al., 2025).

The WGCNA method shows that tolerant maize varieties form large stable network clusters which connect to salt response traits but sensitive lines generate fragmented networks with few metabolite hubs that regulate core functions. The tolerant maize exhibits better saline environment resistance because its metabolic pathways maintain homeostasis at the cellular level.
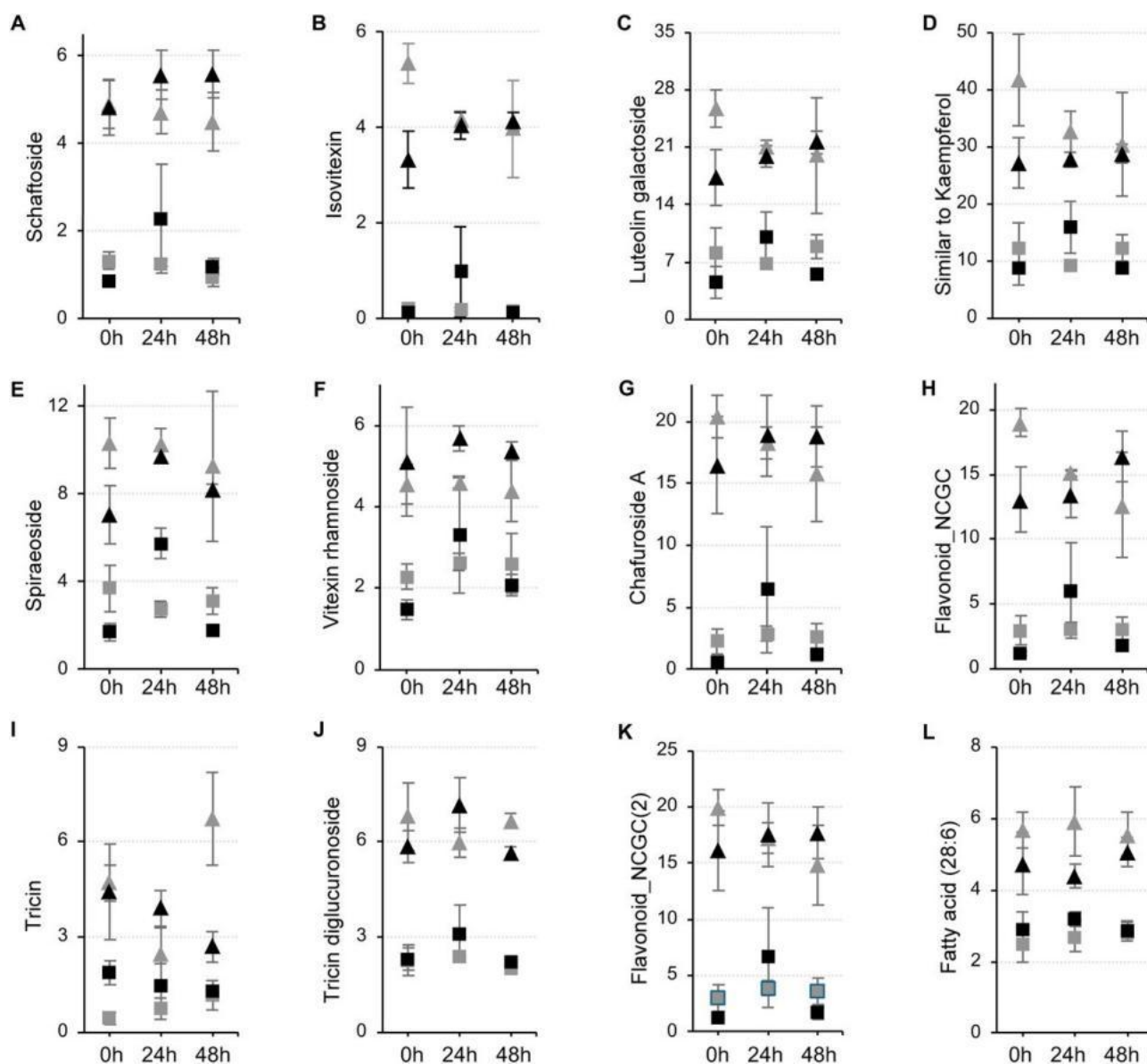

Figure 2 Comparative metabolomic profiling of salt-tolerant and salt-sensitive maize genotypes (Adapted from Brar et al., 2025). Principal component analysis (PCA) and volcano plots highlight metabolite differences such as proline, raffinose, and flavonoids between tolerant and sensitive genotypes

5.3 Metabolite biomarkers offer potential for breeding applications

The main result of comparative metabolomics research leads to the discovery of biomarkers which show strong association with salt tolerance. Plants show salt tolerance through three specific markers which include elevated proline levels and raffinose content and particular lipid patterns that include linolenic acid elevation and phosphatidylcholine modifications (Liang et al., 2021; Wu et al., 2024; Brar et al., 2025). The biomarkers function as (i) screening tools for fast maize germplasm assessment under saline environments and (ii) selection criteria for breeding programs to speed up the creation of tolerant crop varieties. Metabolite biomarkers serve as functional indicators which extend beyond traditional morphological characteristics because they show direct evidence of biochemical salt stress adaptation in plants.

Research that links metabolite biomarkers to genetic mapping studies has identified three essential genes which control stress-responsive metabolic traits through their production of citrate synthase and glucosyltransferase and cytochrome P450 (Liang et al., 2021; Zhao et al., 2023). The research confirms biomarkers function as predictive indicators while revealing specific molecular targets which scientists can use for marker-assisted selection and genomic breeding of salt-resistant maize.

6 Breeding and Translational Applications

6.1 Metabolite-QTL mapping and metabolome-wide association studies link metabolites with genetic loci

The combination of metabolomics with quantitative genetics enables scientists to study how genetics affects salt tolerance in maize through advanced methods. The identification of metabolite quantitative trait loci (mQTLs) reveals genetic regions that control how metabolite levels respond to plant stress conditions. The genetic regions that control proline and sugar and secondary metabolite levels under stress conditions also contain QTLs that affect ion balance and biomass production (Liang et al., 2021; Brar et al., 2025). Scientists use natural diversity analysis with mGWAS to study how specific genetic variations called SNPs affect metabolite variations which helps them identify exact genetic influences on physical traits. The mGWAS research has identified three genes which encode citrate synthase and glucosyltransferases and cytochrome P450s that show strong links to both metabolite levels and salt tolerance under osmotic stress (Liang et al., 2021; Brar et al., 2025). The research methods allow scientists to connect genetic data with metabolic features which results in the identification of precise targets for developing salt-resistant maize varieties.

6.2 Functional validation of candidate genes strengthens causal links

The identification of key genomic regions through QTL mapping and association studies requires functional validation to demonstrate their causal effects. The candidate genes that mQTL or mGWAS identify can be tested using reverse genetics methods and CRISPR-Cas9 genome editing and transgenic overexpression techniques. The study by Liang et al. (2021) shows that P5CS gene overexpression produces more proline which enhances salt tolerance but changes in raffinose biosynthesis and flavonoid pathways create new metabolites that boost salt tolerance in maize. (Brar et al., 2025). The process of functional validation proves the metabolic function of specific genes while showing how transcription factors and enzymes and metabolites interact with each other in a hierarchical manner. The research process leads to the identification of specific molecular targets which scientists can use for developing salt-resistant maize through breeding and genetic engineering.

6.3 Metabolomics-driven strategies accelerate molecular breeding and precision agriculture

Metabolomics shows its potential for translation because it enables researchers to develop sophisticated breeding methods which improve agricultural practices for crop cultivation. The use of metabolite biomarkers in breeding programs enables fast germplasm population selection through marker-assisted and genomic selection methods. The research by Liang et al. (2021) shows that using metabolomic data with genomic prediction models results in enhanced salt tolerance predictions for untested genotypes. (Brar et al., 2025). Molecular design breeding requires basic knowledge of metabolic pathways to introduce specific alleles that control metabolite production through targeted editing or introgression techniques.

Metabolomics helps precision agriculture through stress onset signature detection which enables early warning systems for prompt action. Scientists monitor maize under salinity stress through real-time field assessments by tracking metabolic markers in leaf tissues and root exudates using non-invasive methods. The application of metabolomics-based methods accelerates salt-resistant maize variety development which supports environmentally friendly farming practices and food security needs in areas where salt levels continue to rise (Liang et al., 2021; Brar et al., 2025).

7 Case Studies in Metabolomics of Maize Salt Stress

7.1 Metabolomic profiling of salt-tolerant maize revealed the central role of osmoprotectants

The metabolic analysis of salt-tolerant maize reveals that proline and raffinose function as vital protective compounds which safeguard the plant. The LC-MS analysis of tolerant and sensitive inbred lines under saline conditions showed tolerant genotypes accumulated more proline and raffinose but sensitive lines did not show

significant accumulation of these compounds. The metabolic adjustment of tolerant maize allowed it to maintain better water retention while keeping its photosynthetic processes active according to Liang et al. (2021), Brar et al. (2025) and Ren et al. (2025). Pathway enrichment analyses further highlighted activation of the glutamate–proline axis, suggesting a preferential allocation of nitrogen toward osmoprotectant biosynthesis as a key adaptive strategy (Brar et al., 2025; Ren et al., 2025). The research findings show that compatible solutes serve as vital metabolic markers which enable scientists to detect salt-resistant maize plants under salt stress conditions.

7.2 Differential accumulation of secondary metabolites contributes to genotype-specific adaptation

The research investigated the impact of salinity stress on maize seedling secondary metabolite production through an individual case study. The UPLC-QTOF-MS results showed tolerant genotypes had elevated levels of flavonoids and phenolic acids and terpenoids but sensitive lines showed no or minimal induction. The antioxidant compounds quercetin and ferulic acid in tolerant maize showed strong links to enhanced antioxidant activity which protected cell membranes from peroxidation and improved survival rates under stress conditions (Brar et al., 2025; Ren et al., 2025). Network correlation analyses revealed that flavonoid accumulation was tightly linked to antioxidant enzyme activity, highlighting the synergy between metabolic and enzymatic defenses. The research demonstrates that particular genetic variations within secondary metabolism systems allow plants to build salt stress tolerance.

7.3 Translation of metabolite markers into breeding programs demonstrates practical feasibility

The present translational research merges metabolomics with genetic mapping to identify metabolite markers which help detect salt tolerance in maize plants. The metabolome-wide association studies (mGWAS) of different maize panels revealed raffinose and linolenic acid as the most important metabolites which correlated with tolerance traits such as shoot biomass and ion exclusion capacity (Liang et al., 2021; Brar et al., 2025). The research team confirmed the candidate genes involved in raffinose family oligosaccharide biosynthesis and lipid desaturation through expression analysis. The researchers successfully added these metabolite markers to breeding programs through selection indices which helped discover new salt-resistant germplasm. The research shows that metabolomic markers serve as useful tools for breeding programs which link scientific research to agricultural development.

8 Future Perspectives and Conclusion

Scientists need to study maize salt stress by tracking metabolic changes that occur at various times and locations. The majority of metabolomic research produces static views of biological systems although stress responses create dynamic changes in metabolism which affect various tissues at different developmental stages. The combination of imaging mass spectrometry and single-cell metabolomics techniques enables scientists to study how different plant tissues such as roots and leaves and reproductive organs distribute resources when exposed to salt stress. The research will examine short-term metabolic changes that lead to long-term adaptive responses through the analysis of their initial signaling pathways. The implementation of these methods will help us understand the mechanisms of salt tolerance development in maize.

Future research needs to focus on combining metabolomics with other omics fields and sophisticated computational systems. The analysis of metabolite variation through transcriptome and proteome and ionome data requires advanced multi-omics methods which have successfully established mQTL and mGWAS to link proline and raffinose and flavonoids to their genetic origins (Liang et al., 2021; Brar et al., 2025). The scientific community continues to face a major challenge in determining cause-effect relationships between correlated events. The identification of salt tolerance drivers needs Mendelian randomization and structural equation modeling and machine learning-based causal inference methods to convert metabolomics from descriptive associations into predictive and mechanistic biology.

Metabolomics provides scientists with an effective method to study the biochemical mechanisms that enable maize plants to adapt to salt stress conditions. The research reveals how tolerant and sensitive genotypes adapt through osmoprotectants and secondary metabolites and lipid remodeling pathways. Research studies have found metabolite biomarkers useful for breeding but genetic mapping and functional validation methods are needed to

establish the genetic basis of these biomarkers. The practical application of metabolomic markers in breeding programs becomes evident through particular examples which link scientific laboratory results to agricultural development. The future development of spatiotemporal metabolomics together with multi-omics integration and computational modeling will enhance metabolomics to become the core foundation of systems biology. The execution of these methods according to plan will speed up the development of salt-resistant maize varieties which will protect sustainable farming practices and global food security because salinization has emerged as a major issue.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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