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Metabolite Profiles of White Maize Kernels under Water-Limited Conditions

Maria Yustiningsih*[1, 3], Diny Dinarti*[2], Bambang Sapta Purwoko[2], and Willy Bayuardi Suwarno[2]

[1] Study Program on Plant Breeding and Biotechnology, IPB University, Indonesia
[2] Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Indonesia
[3] Faculty of Teacher Training and Education, University of Timor, Indonesia

*Corresponding author; email: yyustiningsih@gmail.com; dinyagh@apps.ipb.ac.id

Abstract

White maize is recognized for its superior traits and stress tolerance and serves as an important germplasm source in Indonesia. These superior traits are associated with secondary characteristics, including the composition of metabolite compounds in the plant. This study analyzed and clustered the metabolite profiles of eight white maize genotypes and evaluated the putative functional roles of these metabolites in seed development under water-limited conditions. The samples consisted of seeds from six white maize genotypes from East Nusa Tenggara (NTT) and two check varieties, cultivated with irrigation withheld from 50 days after planting (DAP) until physiological maturity. Metabolite profiling was performed using untargeted liquid chromatography-mass spectrometry (LC-MS). Raw data were annotated using ChemSpider and mzCloud. Relative abundance (peak area %) was analyzed using Microsoft Excel, InteractiVenn, RStudio, and MetaboAnalyst. Metabolite profiling revealed approximately 10–19 putative metabolites per genotype, classified into four major groups: carbohydrates (36.0%), lipids (19.29%), organic acids (17.12%), and secondary metabolites (27.5%). Similarity among genotypes ranged from 45.5% to 81.3%. Two metabolites with variable importance in projection (VIP) scores of 2.0 or higher, (E)-3,4,5-trimethoxycinnamic acid and 1-heptadecanoyl-sn-glycero-3-phosphate, contributed to differentiation among genotypes. The predominant primary and secondary metabolites are presumed to be involved in seed filling, starch accumulation, and putative responses to water limitation. The results suggest that white maize genotypes from NTT could serve as important parental lines and genetic resources for future maize breeding programs.

Keywords: cluster analysis, drought stress, germplasm, LC-MS, multivariate analysis, secondary characters

Introduction

White maize is a significant local variety of maize germplasm in Indonesia and reflects the genetic diversity historically cultivated by local farmers, particularly in regions that continue to uphold traditional agricultural practices (Benrey et al., 2024; Guzzon et al., 2021). Regions recognized for their extensive diversity of local white maize types include Sulawesi, Central Java, East Java, West Nusa Tenggara, and East Nusa Tenggara. Currently, Indonesia has approximately 331 accessions of local maize varieties, most of which have been recorded through core, digital, and participatory collection methods. However, the data compilation largely focuses on agronomic traits (Hosang et al., 2020; Ngongo et al., 2021; Risliawati et al., 2022). Features associated with secondary and physiological characters remain inadequately studied and evaluated.

Local varieties of white maize exhibit several beneficial characteristics, including early maturity, prolonged shelf life, and drought

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stress tolerance (Yulita & Naiola, 2013). Genetic variation in stress tolerance correlates with secondary traits, particularly metabolite composition in plant tissues (Fan et al., 2025). Metabolites are plant-derived compounds that play roles in various physiological processes and are considered complementary to agronomic traits, often closely associated with plant phenotypes (Witt et al., 2012). Metabolite profiles also serve as important indicators in genetic and environmental studies, reflecting an organism's response to genetic variation or environmental perturbations (Salam et al., 2023).

Previous research on maize has examined metabolite levels in vegetative organs, including leaves and roots, and their roles in metabolic pre-adaptation to environmental changes (Sun et al., 2016; Witt et al., 2012; Zhang et al., 2021). However, research on seed metabolite profiles and their variability under water-limited conditions remains limited. Grain yield, the main sink in maize, is more strongly affected by environmental factors than vegetative traits (Obata et al., 2015). Seed development depends on assimilates from the photosynthetically active source organs (leaves). Still, seed sink potential, or its ability to store assimilates, is ultimately determined by the physiological capacity and composition of seed tissues relative to assimilate supply (Medeiros et al., 2021; Obata et al., 2015). Identifying the composition and components of the sink therefore remains essential for modern plant breeding.

The metabolite profile of maize seeds may reflect the plant's physiological and biochemical activities, including the assimilation and transport of carbon, nitrogen, and other metabolites from source tissues (Zhang et al., 2024). Seed metabolite analysis elucidates the metabolic processes that enhance maize nutritional quality and distinctiveness, including the accumulation of starch, proteins, amino acids, and lipids, as well as their modifications in response to environmental influences (Obata et al., 2015; Röhlig et al., 2009). Seed metabolites comprise both primary and secondary metabolites, which serve distinct functional roles in plant growth and development. Primary metabolites, including carbohydrates, amino acids, lipids, and proteins, are generally more abundant in seeds and serve as energy reserves and structural components during seed filling, maturation, and early germination (Obata et al., 2015; Sun et al., 2016). In contrast, secondary metabolites such as phenolic compounds, alkaloids, and terpenoids are typically present in lower amounts and primarily function in defense mechanisms rather than serving as direct energy reserves or structural components. These metabolites nevertheless contribute to seed protection against unfavorable abiotic conditions. In wheat (*Triticum aestivum*), the seed-filling stage is characterized by the accumulation of primary metabolites, including carbohydrates such as raffinose and myo-inositol, which peak at physiological maturity (Teng et al., 2023). In maize, glucose accumulation increases during seed ripening but decreases under environmental stress. Under drought stress, complex carbohydrates such as sucrose and oligosaccharides can be hydrolyzed into simpler sugars such as glucose and fructose to fulfill energy requirements (Li et al., 2024; Wang et al., 2022). The remobilization of carbohydrates and amino acids in seed tissues is typically associated with higher flavonoid production, which protects seeds from oxidative damage. This response may also activate additional secondary metabolism pathways, including phenolic compound production, as part of the plant's adaptive response to environmental stimuli (Baskar et al., 2018). Thus, each genotype is presumed to produce unique metabolite responses based on genetic factors and its agricultural environment (Li et al., 2025; Röhlig et al., 2009). This study aimed to analyze and cluster the metabolite profiles of eight white maize genotypes based on their metabolite compositions and to examine the putative functional roles of these metabolites in seed development under water-limited conditions.

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Materials and Methods

Plant Material and Growth Conditions

The eight maize genotypes used in this study comprised six white maize accessions collected from four high-yielding districts in East Nusa Tenggara, Indonesia, namely Timor Tengah Utara (TTU): TTU03, TTU04, and TTU09; Timor Tengah Selatan (TTS): TTS03; Belu: BL01; and Malaka: ML01; and two open-pollinated check varieties, the drought-sensitive 'Srikandi Putih' (SKP) and the drought-tolerant 'Anoman' (ANM). The experiment was conducted at the Maubeli experimental area in TTU district, East Nusa Tenggara, at coordinates 9°28'20" S, 124°29'01" E and an elevation of 471 m above sea level, from September to December 2023, coinciding with the peak of the dry season in this region. All genotypes were planted in the field at 70 cm × 20 cm spacing with three replications. Fertilization was conducted using two types of fertilizer (Efendi, 2021). Irrigation was applied every two days until the stress treatment began. The water-limited condition began at 50 days after planting (DAP) by completely withholding irrigation and was maintained until physiological maturity (Efendi & Azrai, 2015). Before stress imposition, routine irrigation maintained soil moisture in the maize root zone (3 – 5 cm depth) under clayey soil conditions near field capacity. At the start of the drying period, soil water potential was assumed to range from pF 2.3 to 2.6 (-40 to -60 kPa). As the water-limited period progressed toward physiological maturity, soil water potential was assumed to decline gradually to pF 3.4 – 4.1 (approximately -1500 kPa), approaching the permanent wilting point and thereby subjecting the plants to progressive drought stress (Allen et al., 1998; van Genuchten, 1980; Wang et al., 2007). Self-pollinated maize cobs were harvested selectively, guided by indicators of physiological maturity. Cobs were harvested when visual signs of drying were observed, characterized by a brownish-yellow husk, uniformly drying kernels throughout the cob, and shiny kernels at the tip of the cob, at a kernel moisture content of 25% – 30% (Efendi, 2021). The cobs were manually removed from the plant stalks and dried to 10% – 14% moisture content, following the established postharvest protocols of ICAR-IIMR (https://iimr.icar.gov.in; accessed on November 12, 2023).

Sample Preparation

Self-pollinated maize kernels were carefully cleaned to remove residual particles and pest remnants. The samples were air-dried at room temperature on plastic trays and then oven-dried at 40 °C for three days until a constant weight was reached. The dried samples were ground into a fine powder. Extraction was performed by maceration (Widuri, 2022). One hundred grams of powdered sample were immersed in 1 L of 70% ethanol for three days. The maceration process was repeated three times, using fresh solvent for each iteration, and the samples were stirred daily for 10 min during each cycle. The filtrates were collected using Whatman No. 1 filter paper and concentrated using a rotary evaporator at 40 °C. The concentrated extracts were then dried in a vacuum dryer until a paste-like consistency was achieved. The final extracts were placed in 2 ml glass vials, stored at 40 °C, and prepared for injection into the LC-MS instrument.

Metabolite Profiling

Untargeted metabolite profiling was performed using liquid chromatography–mass spectrometry (LC-MS) at the Laboratorium Terpadu, IPB University. Raw data from the LC-MS analysis were annotated using the online databases ChemSpider (https://www.chemspider.com; accessed on July 25, 2024) and mzCloud (https://www.mzcloud.org; accessed on July 25, 2024). The biological functions and putative roles of the identified metabolites were examined using the National Institute of Standards and Technology database (NIST; https://www.nist.gov/; accessed on December 12, 2024), the Plant Metabolic Network (PlantCyc; https://www.plantcyc.org/; accessed on December 20, 2024), PubChem of the National Center for Biotechnology Information (https://pubchem.ncbi.nlm.nih.gov/; accessed on December 20,

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2024), and the Human Metabolome Database (HMDB; http://www.hmdb.ca/; accessed on December 20, 2024).

Data Analyses

We analyzed relative abundance (peak area %) in Microsoft Excel to determine the dominant metabolite composition in each maize genotype. The metabolite similarity index among genotypes was assessed using InteractiVenn (https://interactivenn.net/; accessed on December 14, 2024), RStudio, and MetaboAnalyst 6.0 (http://www.metaboanalyst.ca/; accessed on December 16, 2024). Multivariate analyses, including biplot Self-Organizing Map (SOM), K-means clustering, Principal Component Analysis (PCA), and Partial Least Squares Discriminant Analysis (PLS-DA), were conducted in MetaboAnalyst 6.0 (http://www.metaboanalyst.ca/; accessed on December 18, 2024) to evaluate metabolite clustering across genotypes and their responses to the test environment.

Results and Discussion

All metabolomic analyses presented in this section are based on metabolites identified in maize kernels developed under water-limited conditions. They are organized to describe overall metabolite profiles, metabolic responses revealed by multivariate analyses, and the putative functional roles of key metabolites. This experimental context provides the basis for interpreting kernel development under water deficit.

Metabolite Profiling Under Water-Limited Conditions

The putative metabolite profiles of the local white maize accessions and check varieties indicated 10 to 19 metabolites per genotype. Accession TTU09 had the highest number of detected metabolites (19) and the most diverse metabolite profile, whereas TTS03 had the lowest number (10). TTS03 and ML01 presented simpler profiles without unique compounds. Among the check varieties, ‘Srikandi Putih’ (SKP) and ‘Anoman’ (ANM) differed in metabolite composition. ‘Srikandi Putih’ had more metabolites (15) than ‘Anoman’ (13), indicating a more diverse and extensive metabolite profile. Table 1 summarizes variation in metabolite profiles among the genotypes, and Figure 1 presents the overall composition of seed metabolites, which consisted primarily of four major compound groups: carbohydrates (36%), lipids (19.29%), organic acids (17.12%), and secondary metabolites (27.5%).

A comparative analysis of metabolite profiles across genotypes under water-limited conditions indicated that accessions TTS03 (TTS) and ML01 (Malaka) showed the highest similarity (81.3%; not significant; Table 2). Accessions BL01 (Belu) and TTU03 (TTU) also showed high similarity (80.0%). These findings indicate close biochemical similarity within these local accession pairs. The lowest similarity values were observed between TTU09 and ‘Anoman’ (ANM) (45.5%) and between TTU09 and TTU04 (50.0%), highlighting marked differences in metabolite composition among these genotypes. The two check varieties showed different levels of similarity to the local accessions. ‘Anoman’ showed the highest similarity with TTU04 (71.4%) and TTU03 (66.7%), whereas ‘Srikandi Putih’ showed the highest similarity with TTU09 (70.0%) and BL01 (66.7%). The similarity between ‘Anoman’ and ‘Srikandi Putih’ was 52.6%, one of the lowest in this experiment, indicating markedly different metabolite compositions. Similarity between the check varieties and the local accessions ranged from 45.5% to 71.4%, indicating multiple common metabolites among the genotypes. Complex carbohydrates, including hexitol, and phospholipids such as 1-linoleoyl-sn-glycero-3-phosphoethanolamine were consistently identified in most genotypes. The amino acid derivative 4-oxoproline was detected only in ‘Anoman’, whereas the phenolic compound p-coumaric acid (together with glutamic acid) was detected only in TTU04; these compounds were absent from ‘Srikandi Putih’ and the other local accessions.

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Table 1

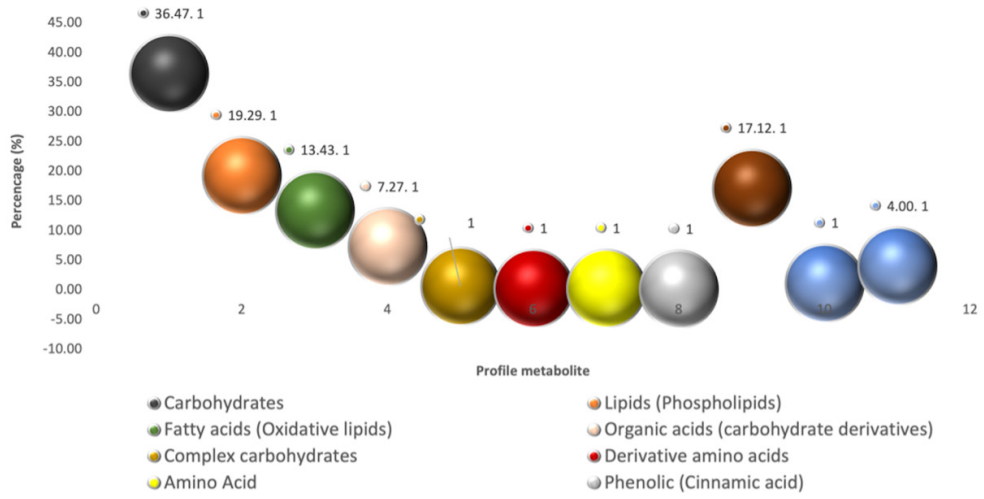
Metabolite Classes in Eight Maize Genotypes Under Water-Limited Conditions

Genotype	Total metabolites	Primary metabolites	Secondary metabolites	Specific metabolites
TTS03	10	Carbohydrate (1); Organic acid (3)	Fatty acid and derivate fatty acid (2); Phospholipid (2); Oxylipin (1); Others (1)	–
ML01	12	Carbohydrate (2); Organic acid (3)	Fatty acid and derivate (2); Phospholipid (2); Oxylipin (1); Others (2)	–
BL01	15	Carbohydrate (2); Organic acid (3)	Fatty acid (3); Phospholipid and derivate (4); Oxylipin (1); Phenolic (1); Others (1)	–
TTU03	12	Carbohydrate (2); Organic acid (3)	Fatty acid (1); Phospholipid (3); Phenolic (1); Oxylipin (1); Others (1)	–
TTU04	12	Carbohydrate (2); Organic acid (3)	Fatty acid (1); Phospholipid (2); Oxylipin (1); Sugar alcohol (1)	Glutamic acid (amino acid); p-Coumaric acid (phenolic)
TTU09	19	Carbohydrate (1); Organic acid (3)	Fatty acid (3); Derivate fatty acid (2); Phospholipid (3); Lipid Complex (1); Phenolic (1); Terpenoid (2); Others (2)	Trimethoxy cinnamic acid (phenolic)
ANM	13	Carbohydrate (2); Organic acid (3)	Fatty acid (2); Phospholipid (2); Oxylipin (1); Others (2)	4-Oxoproline (amino acid derivative)
SKP	15	Carbohydrate (1); Organic acid (3)	Fatty acid (5); Phospholipid (3); Oxylipin (1); Others (2)	–

Note. Values in parentheses indicate the number of putative metabolites. (–) indicate no genotype-specific metabolite detected. Others = putative metabolites not assigned to the listed classes. All samples were collected under water-limited conditions (irrigation withheld 50 days after planting [DAP]). ANM and SKP were check varieties.

Figure 1

Metabolite Profile of Eight Maize Genotypes under Water-Limited Conditions



Note. Colour and circle coding is used solely to distinguish compound categories in the legend and does not represent quantitative data values.

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Several metabolites were consistently identified across nearly all genotypes, including the carbohydrate D-sucrose and three organic acids (citric acid, malic acid, and D-gluconic acid), which are assumed to be fundamental to white maize physiology. Genotypes with similar metabolite profiles or biochemical compounds indicate shared metabolic pathways or regulatory mechanisms (Riedelsheimer et al., 2012; Röhlig et al., 2009). These results align with previous maize metabolomics studies, which indicate that carbohydrate-related metabolites detected in kernels, including sugar alcohols, primarily reflect carbon metabolism and reserve accumulation during grain development (Medeiros et al., 2021; Röhlig et al., 2009). Metabolites unique to particular genotypes, including oxoproline and p-coumaric acid, are proposed to be linked to genotype-specific responses to environmental stress (Mahatma et al., 2021; Xie et al., 2023). Several studies on cereals, including maize, have confirmed phenolic metabolites, carotenoids, and proline derivatives in response to abiotic stress (Mishra et al., 2025). The detection of oxoproline and other amino acid derivatives under drought stress also suggests involvement of stress-related metabolic pathways linked to oxidative stress regulation and amino acid metabolism in maize (Shafiq et al., 2022). However, the comparative analysis reveals variability in genotype responses to the water-limited environment; therefore, it is crucial to cluster and classify both compound-specific and genotype-specific responses, rather than depending exclusively on the presence of specific metabolites.

Metabolic and Genotype Responses to Water-Limited Conditions

Cluster analysis was used to categorize genotypes based on similarities in metabolite accumulation patterns under water-limited conditions. The PCA biplot shows the distribution of maize accessions by metabolite profile under water-limited conditions (Figure 2A). The first principal component (PC1) explained 66.1% of the total variation in metabolite intensity, indicating that this axis captured most of the differences in metabolite content among accessions. PC2 contributed an additional 17.1% of the variation, distinguishing accessions by specific metabolite characteristics.

The PCA biplot suggests that certain metabolites may serve as markers to distinguish maize accessions by their response to the test environment. Genotypes close together on the biplot share similar metabolite compositions, likely reflecting similar genetic backgrounds or analogous environmental responses. Accessions farther apart along PC1 and PC2 likely differ in metabolic response and produce distinct metabolite profiles, consistent with the metabolite similarity analysis. TTU09 is farthest

Table 2

Proportion and Similarity of Metabolite Profiles Across Genotypes

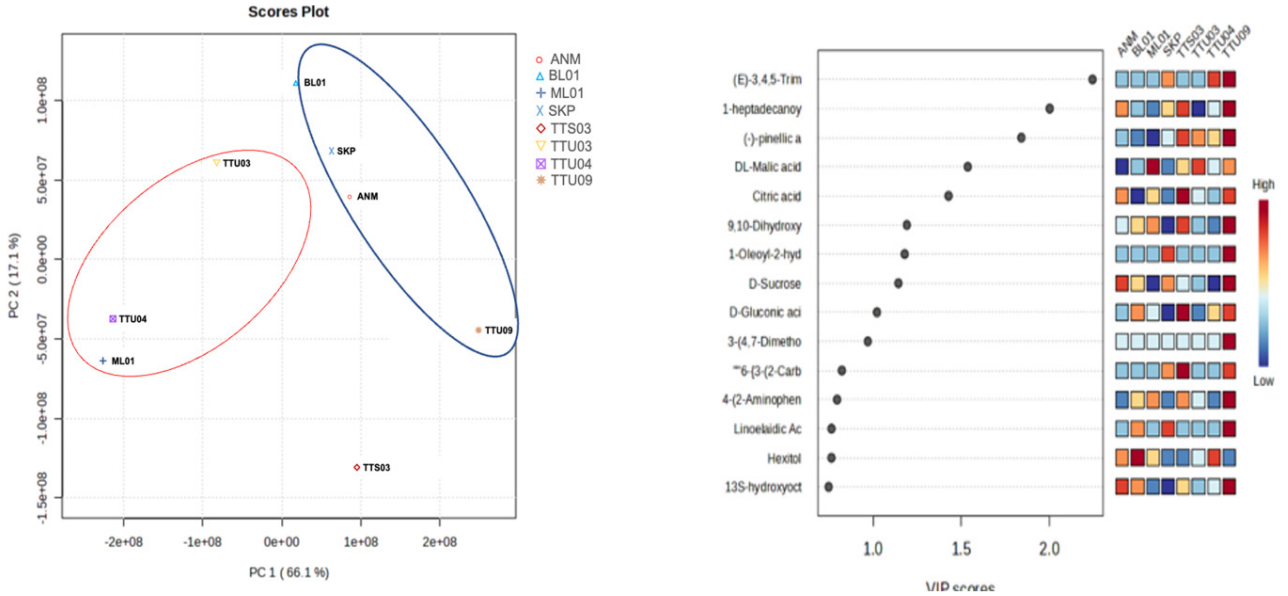
Genotype	TTS03	ML01	BL01	TTU03	TTU04	TTU09	ANM	SKP
TTS03	100							
ML01	81.3 ns	100						
BL01	52.6 **	66.7 *	100					
TTU03	62.5 **	68.8 *	80.0 ns	100				
TTU04	56.3 **	62.5 **	52.9 **	64.3 **	100			
TTU09	65.0 **	61.9 *	61.9 **	55.0 **	50.0 ***	100		
ANM	58.8 ***	64.7 **	64.7 **	66.7 **	71.4 **	45.5 ***	100	
SKP	61.1 ***	57.9 **	66.7 **	58.8 ***	52.9 **	70.0 *	52.6 ***	100

Note. Values are proportions and similarities (%) of metabolites between genotype pairs. * = significant at = 0.05; ** = significant at = 0.01; *** = significant at = 0.001; ns = not significant.

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Figure 2

Principal Component Analysis Biplot and Partial Least Squares Discriminant Analysis of Metabolite Profiles



Note. (A) PCA biplot of the eight maize genotypes. (B) Important features identified by partial least squares discriminant analysis (PLS-DA), ranked by variable importance in projection (VIP) score. Colour coding in the PCA biplot is used solely to differentiate genotypes in the legend and does not indicate quantitative data values.

from the other genotypes in the biplot and has a relatively low average similarity of 45% – 65%, indicating a unique metabolite profile. Interestingly, TTU09 is also distant from the check variety 'Srikandi Putih' (SKP) in the PCA biplot, even though the two share 70% similarity. This inconsistency may reflect major metabolites that strongly influence the PCA vectors, even when overall similarity is high (Xu et al., 2024). A similar pattern was observed for TTS03 and ML01, which have the highest similarity (81.3%) but differ in their positions in the PCA plot. This may indicate that metabolites with high variance strongly influence genotype positioning in the multivariate space. Metabolites with high area % may have a major impact on separation and clustering patterns in PCA (Panda et al., 2018; Worley & Powers, 2012).

Partial Least Squares Discriminant Analysis (PLS-DA) was performed to identify the metabolites that contribute most to genotype variation, as illustrated in Figure 2B. Several metabolites were crucial in differentiating the eight maize genotypes and showed elevated variable importance in projection (VIP) scores, particularly the cinnamic acid derivative (E)-3,4,5-trimethoxycinnamic acid, the glycerophospholipid 1-heptadecanoyl-sn-glycero-3-phosphate, and the unsaturated fatty acid pinellic acid. Of these, (E)-3,4,5-trimethoxycinnamic acid and 1-heptadecanoyl-sn-glycero-3-phosphate reached VIP scores of 2.0 or higher, indicating a major role in genotype differentiation (Zhang et al., 2024), whereas pinellic acid scored slightly lower (about 1.8). Conversely, metabolites such as the disaccharide D-sucrose and the organic acid citric acid had lower VIP scores, indicating a more universal metabolic function. These compounds were detected across a variety of genotypes and did not differ markedly among any specific accession. However, metabolites that contribute substantially to PLS-DA are not necessarily specific to a single genotype; they can be present in all genotypes but at widely

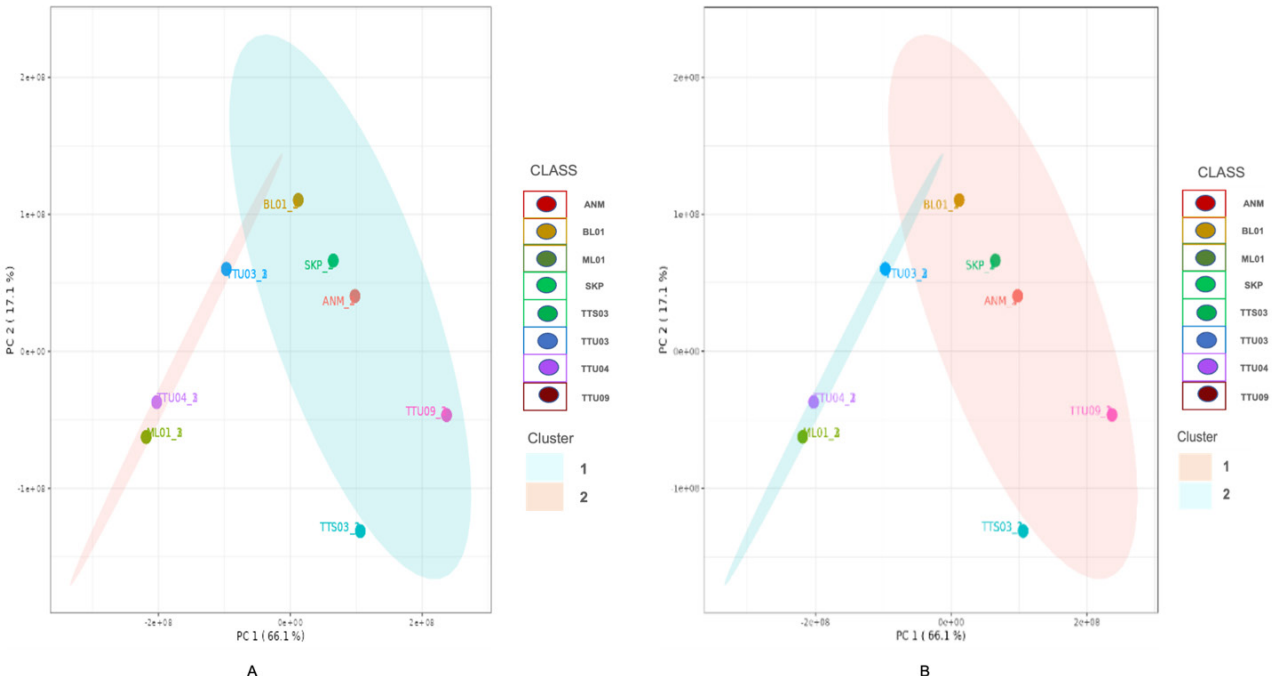
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differing concentrations (Boutchouang et al., 2024; Kalivodová et al., 2015). Our results indicate that (E)-3,4,5-trimethoxycinnamic acid was present exclusively in TTU09. In contrast, the phospholipid 1-heptadecanoyl-sn-glycero-3-phosphate was detected in most accessions, excluding TTS03 and ML01, but with varying peak area levels. D-Sucrose and citric acid, with intermediate VIP values, were present in all genotypes (except TTU04) and had nearly equal peak area values.

Additional clustering analyses used the Self-Organizing Map (SOM) biplot and K-means clustering to validate and further investigate the grouping of maize accessions in response to the test environment (Han et al., 2019). Both methods revealed two primary clusters, indicating clear variation in metabolite composition among the accessions. The SOM biplot grouped the maize genotypes into two clusters, represented by light blue and pink ellipses (Figure 3A). The first cluster consisted of accessions BL01, 'Srikandi Putih' (SKP), 'Anoman' (ANM), TTU09, and TTS03, whereas the second cluster comprised TTU03, TTU04, and ML01. Accessions in the first cluster were more dispersed, indicating greater heterogeneity in their metabolic responses to the water-limited environment. Accessions in the second cluster were more tightly grouped, suggesting more similar metabolite compositions among those genotypes. The clustering was consistent with the PCA results, which showed broader dispersion of accessions with variable responses within the larger grouping. The K-means clustering biplot also identified two primary clusters, with minor differences in accession distribution compared with the SOM results (Figure 3B). In the K-means output, the pink cluster displayed a wider dispersion on the biplot, whereas the light blue cluster showed a more linear, diagonal arrangement, indicating greater similarity in metabolite composition among its constituent accessions. The resulting clusters of genotype variation and metabolic

Figure 3

Self-Organizing Map Biplot and K-Means Clustering of Maize Genotypes Based on Metabolite Profiles



Note. (A) Self-Organizing Map (SOM) biplot. (B) K-means clustering. Each colour represents a different cluster and is used solely to distinguish clusters for easier interpretation of the grouping results.

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response are consistent with the PCA and PLS-DA analyses and may enhance the reliability of genotype classification based on metabolite profiles.

Functional Relevance of Metabolite Profiles

Metabolite identification and genotypic clustering reveal varied responses of maize accessions to the water-limited environment, underscoring the need to understand the roles and potential functions of individual metabolites. Figure 1 and Table 1 show that the metabolite composition of maize kernels consists primarily of primary metabolites (72.5%), including D-sucrose, D-gluconic acid, DL-malic acid, and citric acid, and secondary metabolites (27.5%), namely (E)-3,4,5-trimethoxycinnamic acid, 13S-hydroxyoctadecadienoic acid, p-coumaric acid, 4-oxoproline, and (-)-pinellic acid.

Primary metabolites are closely linked to seed filling and maturation, particularly the biosynthesis of starch and protein reserves in seed tissues (Wang & Komatsu, 2017). These results align with earlier research on wheat and barley, which indicates dynamic changes in primary metabolite levels, including disaccharides, organic acids, amino acids, and lipids, throughout seed development (Szablińska-Piernik & Lahuta, 2023). In wheat, approximately 30 primary metabolites are associated with agronomic traits, seed stability, and grain weight during development (Shi et al., 2020; Yin et al., 2024). In maize, researchers have detected more than 50 primary metabolites, with sucrose dominating the seed carbohydrate profile and reflecting the efficiency of carbon translocation from source to sink (Jiang et al., 2023).

The substantial accumulation of sucrose in the kernels indicates active translocation of assimilated carbon from source tissues to developing seeds, where it serves as a primary energy source for growth and starch deposition. Beyond supporting metabolic activity during maturation, sucrose also serves as a precursor for various metabolic pathways (Pegler et al., 2023). Increased sucrose concentrations may also reflect an adaptive physiological response to environmental stress (Horacio & Martinez-Noel, 2013). Primary metabolites, specifically carbohydrates, lipids, and organic acids, are essential for key physiological processes. Lipid metabolites, including phospholipids and complex fatty acids, maintain cellular membrane integrity and provide energy for early germination (Li-Beisson et al., 2013). Similarly, organic acids such as malic and citric acids are vital intermediates in the tricarboxylic acid (TCA) cycle and facilitate energy production during the seed-filling stage (Ma et al., 2017). Secondary metabolites, such as phenolic compounds and flavonoids, enhance membrane stability and signal responses to environmental stress (Al-Khayri et al., 2023; Tiozon et al., 2022). In this study, 4-oxoproline was detected only in the drought-tolerant check variety ‘Anoman’ (ANM), and p-coumaric acid only in the high-yielding accession TTU04. While these compounds may support stress adaptation, their mechanisms remain speculative. Future research involving targeted metabolomic profiling and enzyme activity assays, including well-watered controls, is required to validate these metabolic pathways across broader populations.

Conclusions

Metabolite profiles of eight white maize genotypes varied under water-limited conditions. Each genotype contained 10 to 19 putative metabolites, of which 72.5% were primary and 27.5% secondary. Primary metabolites are presumed to be involved in seed development, starch production, and reserve accumulation, whereas secondary metabolites may be involved in putative responses to water limitation. These findings provide a basis for selecting white maize genotypes from East Nusa Tenggara, Eastern Indonesia as parental lines in breeding programs, pending validation with targeted metabolomics and well-watered controls.

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